Induction of Antiviral Neutralizing Antibodies in Humans and Animals

Description

The invention relates to immunogenic constructs and to a pharmaceutical agent for inducing humoral neutralizing immune responses to viral infections and to a kit for the detection of antibodies and viral antigens based on said immunogenic constructs and induced antibodies. The invention also relates to a method for inducing an antibody response and to a method for the passive immunization of an organism using neutralizing antibodies obtained using the above-mentioned immunogenic constructs and to a bioassay for detecting viral infection.

Since first described in the early eighties of the last century, infections with human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS) resulting therefrom have developed to become one of the greatest global viral epidemics. In 2002 alone, five million people worldwide became infected with HIV, including 800,000 children less than 15 years of age, and 3.1 million people died as a consequence of HIV infection and/or AIDS. Thus, the total number of HIV-infected individuals amounts to more than 42 millions (1). To date, a total of more than 20 million people have died from HIV/AIDS. It has been estimated that 45 million new infections in 2010 and about 70 million dead persons in 2020 would have to be expected if no effective countermeasures are taken (2).

A number of therapies for viral diseases such as HIV infections or AIDS are known in the prior art. For example, monotherapies using inhibitors of reverse transcriptase, such as AZT, combination therapies, especially using reverse transcriptase inhibitors and protease inhibitors such as AZT and nevirapin, or a combination therapy of antiretroviral substances with immunomodulators and other substances such as interferon, interleukins and/or thymostimulin have been described. By virtue of such therapies it is possible to stabilize the physio-

logical condition of HIV-infected individuals. However, a cure in a classical sense, i.e., elimination of the virus, is not possible. For this reason, efforts have been made time and again in order to provide new and effective pharmaceutical agents which, in the form of a prophylaxis or therapy, would activate the humoral or cellular immune response in an infected organism in such a way that viruses would be neutralized or destroyed.

Those skilled in the art are well aware of the fact that immunization can be effected in an active or passive fashion. In artificial active immunization, application of well-defined antigens is effected, resulting in actively acquired immunity through formation of antibodies or cellular immunity. In passive immunization, direct application of antibodies or immune cells is effected, resulting in a passively acquired immunity. For example, artificial active immunization of an organism against viruses can be effected by means of inactivated viruses, viral proteins, or peptides derived from viruses, inducing e.g. neutralizing antibodies. Neutralizing antibodies for various viral diseases have been described and can be induced in an organism by administering particular antigens, especially viral proteins. Such activation or induction of an immune response is called inoculation. For example, those skilled in the art are familiar with vaccines against influenza virus, smallpox virus, measles virus and polio virus. Induction of neutralizing antibodies against porcine endogenous retroviruses (PERVs) has also been obtained (Fiebig et al., 2003); however, the effectiveness of this inoculation strategy could not be established because of lacking animal models wherein PERVs could grow. For immunization, the complete ectodomain of the transmembranous envelope proteins of the above viruses has been applied. Even when using analogous envelope proteins or binding structures, it has not been possible to successfully transfer this immunization strategy to other viruses such as HIV.

In combatting HIV infections, it has initially been assumed that administration of attenuated or destroyed viruses, optionally in association with adjuvants, could trigger a humoral - e.g. via antibodies - or a cellular immune response in the organism, e.g. via T cells. Especially a short time after the AIDS disease had be-

come known, attempts of inducing an immune response via administration of or vaccination with a destroyed or attenuated virus or envelope proteins thereof were massively pushed forward because this technique allowed to achieve good results in other viral diseases. However, it became soon apparent that this method would not allow induction of a desired antibody response, because the viruses used for testing were growing on foreign cells and the repulsion of said viruses was based on foreign cellular antigens in the virus envelope. In addition to cellular antigens, especially whole-virus vaccines against HIV may include structures developed by the virus to inhibit the immune system, such as immunosuppressive domains or masking carbohydrates, so that the vaccines that have been developed fail to achieve optimum effects.

At present, there is no vaccine capable of inducing an effective immune response to HIV infection (3). In general, two strategies for inducing an immune response can be distinguished. Peptide/protein vaccines, but also DNA vaccines, induce a humoral immune response (4), i.e., production of antibodies to protect from infectious agents. Attenuated viruses, as well as DNA and lipopeptide vaccines, induce production of pathogen-specific protein/peptide sequences in the host cells, which are subsequently presented as CTL epitopes in the MHC (3,4), resulting in a cellular immune response mediated by cytotoxic T lymphocytes. Up to now, high-ranking scientists indicate that only those vaccines capable of inducing cytotoxic T cells would be suitable for preventing HIV infection (3).

Although the search for a vaccine has developed in this direction during the last years, the prior art describes a number of attempts of inducing a humoral immune response, i.e., formation of antibodies. Here, the point is to induce not only so-called binding antibodies, but also neutralizing antibodies, i.e., antibodies capable of preventing infection. A human, monoclonal antibody (2F5) described in 1993, which has been obtained via a cell culture from an HIV-positive patient, demonstrates that it is possible, in principle, to induce neutralizing antibodies in humans, and even in HIV-infected individuals. This antibody has a virus-

neutralizing spectrum comprising virtually all subtypes of HIV; the antibodies bind to an epitope located at the N-terminal site of the transmembrane passage of the transmembranous envelope protein gp41. It has been possible to demonstrate that the antibodies interact with the ELDKWA epitope. The obvious thing to do was therefore to inject this epitope in the form of a peptide or modified peptide, e.g. in cyclized form, into an organism so as to trigger an antibody response to said epitope and hence to the virus. Thus, J. Tian et al. (2002) suggest linear nonapeptides which have high affinity to the 2F5 antibody. However, it was invariably binding antibodies and never neutralizing antibodies that were induced following immunization with countless forms and modifications of the ELDKWA epitope. There is general agreement in the art that induction of antiviral neutralizing antibodies does not represent a promising option of HIV prevention.

Furthermore, it has been attempted to inject single gp41 epitopes together with other peptides, part thereof chemically bound to each other, assuming that the presence of additional peptide-like structures would result in active formation of neutralizing antibodies in an organism. For example, Parker et al. (2001) suggest extension of the injected domain of the peptide around the flanking regions in the natural state of this epitope in gp41 in such a way that a 16 amino acid peptide is produced, because the latter should be more immunogenic than the actual epitope due to its higher complexity. Ho (2002) believes that the native epitope has rather a β-turn-like than a helical conformation, and that the epitope therefore must be provided to the living organism as a vaccine in the form of a β-turn-like configuration in order to have active antibody production. Similarly, however, such attempts invariably induced binding antibodies in living organisms under laboratory conditions, and never neutralizing ones.

The investigations of Ferrantelli et al. (2003), who used neutralizing antibodies for passive immunization, showed that neutralizing antibodies such as the 2F5 antibody are suitable not only for prevention, but also in therapy, and apart from 2F5, the authors have disclosed further antibodies for passive immunization, such as IgG1b12, 2G12 and 4E10. Owing to its extraordinary immunogenicity,

the V3 loop of the HIV surface protein gp120 is regarded as a promising agent to induce neutralizing antibodies, and the 1b12 and 2G12 neutralizing antibodies are directed against gp120. Other surface proteins such as the above-mentioned gp41 are regarded as promising little success, because fusion with the target cell via said protein is so rapid that an antibody is not capable of interacting with said protein rapidly enough. Apart from the neutralizing antibodies usable in therapy, another therapy has been developed, which is directed against gp41. In this case, peptides are concerned which, being very small molecules, can interact effectively with the structures of gp41 responsible for infection (Weiss et al., 2003). One of these peptides, T-20, includes the above-described ELDKWA sequence.

One crucial drawback of the vaccination strategies set forth above, especially the one against gp120, is that the vaccines being used are incapable of preventing formation of new virus variants or modifications during the course of a viral disease (escape mutants). The neutralizing antibodies formed against the original virus are incapable of interacting with the mutated virus in such a way that successful preventive protection or effective therapeutic treatment of an infected organism would be possible.

The object of the invention was therefore to produce a vaccine that could be used in the prevention, diagnosis and therapy of viral diseases, particularly retroviral diseases.

According to the invention, said object is accomplished by providing an immunogenic construct comprising amino acid sequences selected from a viral transmembranous envelope protein which is associated with the viral membrane via at least one membrane passage and has at least one fusion domain and at least two α-helical structures, the amino acid sequences being selected from

(i) a first region of the protein, located between the membrane passage, and a first α-helical structure, and

(ii) a second region located between the fusion domain and a second α-helical structure.

It should be noted that the combination of the above two regions is a crucial issue.

Also suitable is a construct comprising a DNA encoding the respective amino acid sequence, and in this case as well, it is the presence of the two regions in the coding sequence that matters.

Hence, what is claimed is an immunogenic construct consisting of different amino acid sequences. The claimed amino acid sequences can be selected from a viral transmembranous envelope protein. The precondition to be met by said envelope protein is that it is associated with the viral membrane via at least one membrane passage and has at least one fusion domain and at least two α-helical structures. As is well-known to those skilled in the art, a number of envelope proteins such as GP2, gp20, gp30, gp37, gp160, p15E, HA2, F2, etc. comply with these preconditions. Each of the above-mentioned envelope proteins is a viral transmembranous envelope protein which has at least one membrane passage, one fusion domain and two α-helical structures.

According to the invention, a person skilled in the art is to select two regions of amino acid sequences from the above-mentioned viral transmembranous envelope proteins. The first region is a freely selectable segment of the envelope protein between the membrane passage of said envelope protein and of the first α-helical structure having said envelope protein. The second region to be selected from the above-mentioned envelope protein is the section located between the fusion domain of the transmembranous envelope protein and the second α-helical structure of said envelope protein, and the α-helical structure, the membrane passage and/or the fusion domain can be part of the immunogenic construct. Combination of the above two regions results in the induction of said

neutralizing antibodies. Secondary and tertiary structure investigations of all the above-mentioned and claimed envelope proteins are already available, so that a person skilled in the art can exactly determine the above-mentioned regions from which the amino acid sequences are to be selected, without being restricted to particular regions. An essential feature of the invention is that the immunogenic construct comprises two amino acid sequences, and said two amino acid sequences are to be selected from very specific regions of viral transmembranous envelope proteins.

Surprisingly, it was found that an immunogenic construct comprising at least two amino acid sequences is capable of inducing neutralizing antibodies in an organism and thus can be used as a vaccine, optionally together with adjuvants well-known to those skilled in the art. An immunogenic construct in the meaning of the invention is any agent suited to induce neutralizing antibodies. As a result of such antibody response, antibodies having a neutralizing effect on a particular retrovirus or other virus are provided in an organism. Considering the complexity of the mechanisms involved, neutralization of a virus is understood to be any mechanism inhibiting infection by viruses *in vivo* or *in vitro*, preventing growth thereof in a preventive meaning, or inhibiting further growth of viruses in a therapeutic meaning, or making a combination therapy more effective. That is, the construct according to the invention activates the immune system and induces formation of neutralizing antibodies. Advantageously, the neutralizing antibodies are directed particularly against those viral structures effecting induction thereof, and, according to the invention, cross-reacting antibodies are not excluded.

During an infection with enveloped viruses, including retroviruses, including HIV, interactions between the surface envelope proteins and cellular receptors take place, subsequently giving rise to conformational changes in the transmembranous envelope protein and fusion with the cellular membrane (Fig. 1, Fig. 2). The transmembranous envelope proteins include highly conserved domains which play an important role in the infection process and therefore represent excellent targets for the induction of broadly neutralizing antibodies.

For example, it is possible in the meaning of the invention to directly obtain the membrane-associated viral protein or transmembranous envelope protein from a virus for further use. In particular, the viral envelope protein of gp41 of HIV can be concerned. Among other things, envelope proteins have a component anchored in the membrane, and a component which at least partially and temporarily protrudes outwardly from the membrane. According to the invention, the selected amino acid sequences are preferably derived from the portion protruding from the membrane. The transmembranous envelope protein comprises an ectodomain, an anchor and a cytoplasmic portion, and the ectodomain has a fusion domain, a first α-helical structure, a cysteine loop, and a second α-helical structure. At the C terminus, the second α-helical structure is followed by an anchor domain which anchors the gp41 transmembranous envelope protein in the viral membrane. The selected amino acid sequences, which can be e.g. domains, peptides or recombinant proteins or fragments thereof, can be obtained in various ways by a person skilled in the art, preferably by means of peptide synthesis or preparation using genetic engineering. Instead of using the amino acid sequences, it is, of course, also possible to use the DNA encoding same. For example, the DNA can be packed in a vector, and the corresponding amino acid sequence will be encoded in the cells. Those skilled in the art are familiar with such methods from gene therapy.

According to the invention, an N-terminal region, e.g. in gp41 of HIV, is selected between the fusion domain and the first α-helical structure, which can also be referred to as peptide section. A second peptide is selected from the region between the membrane and an α-helical structure facing the membrane. In the meaning of the invention, "selecting between two regions" implies that at least part of the flanking regions, such as membrane passage, α-helical structure and/or fusion domain, can be constituents of the selected section. One important issue is that the amino acid sequences which, in the meaning of the invention, are selected between the membrane passage and an α-helical structure and between the fusion domain and the other α-helical structure, are used in combina-

tion only and may comprise partial or complete regions of the membrane passage, fusion domain and/or α-helical structure or structures.

The term "α-helical structure facing the membrane" merely describes the relationship of the at least two α-helical structures to the membrane rather than the alignment of said α-helical structure; the α-helical structure facing the membrane has a smaller spatial distance to the membrane if the envelope protein is represented or taken as a linear unfolded structure. Accordingly, the facing structure is spatially closer to the membrane passage of the envelope protein imagined to be linear. Of course, the positions of particular components of the envelope protein can be altered as a result of natural or artificial folding processes.

The amino acid sequences selected from the envelope protein, e.g. peptides or protein domains, which correspond to the regions close to the α-helical structures can be taken from the overall protein, or, once their natural sequence is known, obtained by means of synthesis or genetic engineering. The thus-obtained peptides, recombinant or viral proteins are used as immunogenic construct by administering them to an organism. Using routine tests, measuring e.g. the antibody production or response, the size of the selected amino acid sequences can be determined by a person skilled in the art.

An essential issue to the invention is to select two amino acid sequences from the region of an ectodomain of a, particularly, transmembranous envelope protein of a virus, the first amino acid sequence being selected from the region between the fusion domain and a flanking or spatially separated subsequent α-helical structure and the second amino acid sequence from the region between the membrane of the virus and the next α-helical structure. Synthetic peptides, recombinant proteins, or a DNA encoding said amino acid sequences are produced according to the above amino acid sequences. The selection can be such that the amino acid sequences still comprise regions of the α-helical structure or of the anchor structure anchoring the envelope protein to the membrane.

Apart from HIV, it is, of course, also possible to obtain immunogenic constructs according to the invention from any other enveloped virus, including retroviruses, and also preferred are: FeLV, MuLV, BIV, CAEV, EIAV1, FIV, OMVV, SIVmac, SIVcpz, VILV, RSV, ALV, JSRV, SMRV, SRV, GALV, BLV, HTLV-1, HTLV-2, Marburg virus, Ebola, SARS virus, influenza virus, measles virus, mumps virus and/or HPV-1. All of the amino acid sequences, peptides or recombinant proteins or viral proteins thus obtained can be used as immunogenic construct to generate neutralizing antibodies against these viruses, and, according to the invention, cross reactivity cannot be excluded, so that e.g. an immunogenic construct obtained e.g. from MuLV or in an entirely artificial fashion may also have an effect against HIV or FeLV, for example. In a preferred fashion, however, the neutralizing antibodies induced by the immunogenic construct are directed against those viruses from which the respective peptides are obtained. The immunogenic constructs can also be recombinant proteins constituted of portions of the transmembranous envelope protein of a virus and two inventive domains of another virus, i.e., a so-called hybrid. Furthermore, DNA corresponding to said peptides or recombinant proteins of the above viruses is used for immunization. Also preferred is a combination of DNA immunization and subsequent immunization with peptides or recombinant proteins or in multiple succession. For example, the neutralizing effect of the antibodies on enveloped viruses, including retroviruses, with respect to their prophylactic potential becomes apparent as inhibition of viral infection, inhibition of syncytium formation, inhibition of fusion between a virus and a target membrane, as reduction or stabilization of the growth rate of viruses in an organism, or in another way. For example, the neutralizing effect with respect to their therapeutic effect may consist in an improved effectiveness of particular antiviral medications as a result of induction or application of the antibodies, e.g. as a desirable side effect, or in a reduction of the number of side effects of such medications as a result of dose reduction. That is, the effect of the antibodies in the meaning of the invention is not restricted to elimination of viruses, but rather encompasses the entire spectrum of advantageous effects in therapy or prophylaxis.

Said at least two peptides or recombinant proteins or the corresponding DNA thereof can be used alone or in combination, optionally together with other antigens of interest. For example, they can be used together with other antigens in the form of physical mixtures or chemically bound to each other, with or without a spacer molecule. Of course, said two inventive peptides or recombinant proteins can also be bound to each other by chemical or physical means. It is also possible to couple the peptides with carrier peptides, proteins or carrier substances. In this event, albumins, KLH, MAP and other proteins known to those skilled in the art for their immunogenic capability can be concerned, for example; also preferred as carrier substances are thyroglobulin and BSA. These proteins can be bound via non-peptide bonds, e.g. via a disulfide bridge, or bonds via calcium ions, but binding via peptide bonds is also possible. Obviously, the peptides could also be substitution, deletion and addition analogs of the peptides according to the invention.

According to the invention, said viruses can be all those viruses which have a membrane undergoing association with envelope proteins or similar structures, and these structures are required to have at least one ectodomain containing a fusion domain and/or an α-helical structure. The membrane of the viruses can be any structure that comprises lipids, provided it enables binding to proteins.

According to the invention, incorporation of the immunogenic construct in an organism can be effected in any way that enables contacting the immune system with the construct such that an antibody response would be induced. For example, this can be done in such a way that the immunogenic construct is ingested on the oral or rectal, enteral or parenteral route, or by direct injection into the body, e.g. into selected organs such as the spleen, or into blood vessels. Also preferred is oral vaccination or vaccination by injection. Preferred injections are intradermal, subcutaneous, intramuscular or intravenous injections. Of course, it is also possible to provide the immunogenic construct in the form of an aerosol which is inhaled by an organism, preferably by a human patient. Thus, DE 198 51 282 A1 describes a method for the application of antigens on mucous

membranes, which hereby is incorporated in the disclosure of the present invention. Further applications will be illustrated in the following discussion in the context with the method according to the invention. The DNA used for immunization is applied using e.g. so-called jet vaccinators, which introduce the DNA by means of gold beads into the skin, or syringes which allow introduction of the DNA under the skin or into a muscle.

Surprisingly, it was found that the synergistic action of at least two amino acid sequences, e.g. two peptides or recombinant proteins - which are parts of well-defined domains and located between other well-defined domains - from an envelope protein of a virus results in a simple immunogen which is effectively provided. As a result of the synergism of the two peptides or recombinant proteins or of the corresponding DNA, at least one epitope is presented to the immune system in such an effective fashion that neutralizing antibodies against viruses are generated.

The construct of the invention is artificial in a sense that it does not represent a naturally occurring complete envelope protein, i.e., the natural complete envelope proteins are not included in the teaching of the invention. When used as vaccine, naturally occurring transmembranous envelope proteins isolated from a virus or the corresponding soluble ectodomains of the transmembranous envelope protein (except for HIV) can be employed. In the simplest case the construct is constituted of two amino acid sequences not bound to each other chemically or otherwise. However, it can be advantageous when the amino acid sequences are associated directly or via a linker or a carrier. In a likewise preferred fashion the selected amino acid sequences are transferred from an envelope protein into another viral envelope protein or fragments thereof by addition or substitution or other methods well-known to those skilled in the art. Consequently, the carrier of the selected amino acid sequences of a virus can be the transmembranous envelope protein of another virus.

To increase the protective or therapeutic effect of the peptides or recombinant proteins of the invention, i.e., essentially increasing the induction of neutralizing antibodies, the immunogenic construct or any agent that can be produced therefrom, particularly the inoculant, the vaccine, are added with adjuvants. For example, well-known adjuvants for human vaccines are aluminum compounds such as aluminum hydroxide or aluminum phosphate. Aluminum hydroxide is a component of numerous inactivated or subunit vaccines and, *inter alia*, in hepatitis B virus vaccines, and is therefore well-known to those skilled in the art. Further well-known adjuvants include MF59 in Fluad®, for example. In the meaning of the invention, however, any substance enabling, enhancing or modifying a specific immune response to the peptides or proteins of the invention when administered simultaneously together with same or after a short or prolonged interval of time is an adjuvant in the meaning of the invention. Thus, for example, coapplication of egg albumin in complete Freund's adjuvant may possibly give rise to an increased cell-mediated immunity and thus support the effect of neutralizing antibodies. Further adjuvants of interest are e.g. saponines such as QS21, muramyl dipeptide, muramyl tripeptide and compounds having a muramyl peptide core, proteins such as γ-interferon and TNF, or phosphatidyl choline, squalene, or the polyols which are well-known to those skilled in the art. The same applies to the DNA used for immunization. In this case as well, the DNAs which have immunostimulatory properties themselves, or encode a protein with an adjuvant effect, including cytokines, can be applied in parallel or in a construct.

The immunogenic constructs present either as a recombinant protein or as a synthetic peptide, with the aforementioned modifications, can also be used in combination with other vaccines, including even commercially distributed vaccines, irrespective of whether the other vaccine is directed against the same virus or against other viruses (combined vaccination).

Some of the terms in the context with the teaching according to the invention will be explained and defined below:

The term "antigen" as used herein refers to a compound including one or more domains to which an immune response is desired. The binding sites of the antibodies in these domains are referred to as epitopes. This definition also encompasses complex mixtures of antigens, such as destroyed cells, bacteria or viruses, respectively fractions thereof, each one in association with the inventive peptides, recombinant proteins or DNA used in immunization.

The term "admixing" as used herein refers to the addition of an excipient to the inventive peptides, recombinant proteins, complex mixtures and/or adjuvant of interest, e.g. by mixing dry reagents or mixing a dry reagent with a reagent in solution or suspension, or mixtures of aqueous formulations of reagents.

The term "excipient" as used herein refers to a non-therapeutical carrier added to a pharmaceutical composition, which carrier is pharmaceutically acceptable, i.e., non-toxic to recipients at applied dosages and concentrations. Suitable excipients and formulations thereof are well-known to those skilled in the art, e.g. from Remington's Pharmaceutical Science, 16th edition, 1980.

"Vaccine" as used herein refers to a formulation of the inventive peptides or recombinant proteins or the DNA usable in immunization, optionally in combination with another antigen or another DNA, intended to furnish a prophylactic, therapeutic or diagnostic reaction inside or outside a host when exposing the host to said antigens. Exemplary vaccines comprise vaccines against diseases such as HIV/AIDS, SARS, FeLV and others.

The expression "therapeutic amount" as used herein refers to an amount which prevents or improves the symptoms of a disorder or of a responsive, pathologically physiological condition. In specific embodiments of the present invention the amount administered is sufficient to trigger an immune response essentially preventing or inhibiting infection or spreading of an infectious agent such as SARS, HIV or FeLV in a recipient.

The amount of immunogenic construct to be used in a healthy individual in the event of prophylaxis or in a patient in the event of therapy is formulated and the dose established according to common medical practice, taking account of the disorder to be treated, the condition of the particular patient, the site of administration, the method of administration, and other factors well-known to the attending physicians. Similarly, the dose of the vaccines administered will depend on the properties of the antigen used, those of the immunogen, e.g. on its binding activity and *in vivo* half-life in plasma, and on the concentration of the antigen in a formulation, the route of administration, site and rate of dosage, the clinical tolerance of the respective individual (human and animal), the pathological affection of the patient and the like, as is well-known to physicians and other persons skilled in the art. In general, dosages of about 0.1 to 1000 mg per individual and administration are preferred. Also, varying dosages can be used in a series of consecutive vaccinations; the attending physician can administer a first vaccination which is subsequently boosted with relatively low doses of adjuvant, in which case preferred ways would be protein-protein, peptide-peptide, protein-peptide or vice versa, and DNA-protein/peptide. In addition, reference is made to the following explanations regarding the method according to the invention.

For example, injections (intramuscular or subcutaneous, or into blood vessels) are envisaged as a preferred route for therapeutic administration of vaccines, e.g. encapsulated or carrier-bound vaccines, although supply in the form of an aerosol, via catheters or surgical tubes can also be used. Alternative routes comprise suspensions, tablets, capsules and the like for oral administration, commercially available nebulizers for liquid formulations and inhalations of lyophilized or aerolyzed compounds, and suppositories for rectal or vaginal administration. For example, liquid formulations can be employed starting from powder formulations. For prophylactic immunization, injections of said proteins, peptides and DNA are envisaged. The suitability of the selected inoculation parameters, e.g. dose, regime, selection of adjuvant and the like can be determined by taking serum aliquots from a patient, i.e., human or animal, and testing for antibody titers during the course of the immunization protocol. Alternatively

and concomitantly, it is possible to determine the amount of T cells or other cells of the immune system in a conventional manner so as to obtain an overall view of the immunologic constitution of the patient. In addition, it is possible to observe the clinical condition of a patient for a desired effect, e.g. an anti-infectious effect. For example, as HIV or other diseases can be associated with other diseases, additional co-monitoring of the latter is also possible. In those cases where insufficient immunization is achieved, the patient can be boosted with further vaccinations, and the inoculation parameters can be modified such that an improvement of the immune response can be expected, preferably increasing the amount of peptide or antigen and/or adjuvant, complexing the peptides with a carrier or conjugating the latter to an immunogenic protein, or changing the route of administration.

In general, both aqueous formulations and dry peptides or adjuvants can be mixed with an excipient to provide a stabilizing effect prior to treatment, e.g. by a solvent. An aqueous solution of a peptide can be a peptide in suspension or a solution.

The inventive recombinant protein, the DNA construct and/or the peptide can be incorporated in a solution together with a preservative. Examples of suitable preservatives of suspensions or solutions include phenol, benzyl alcohol, m-cresol, methylparaben, propylparaben, benzalkonium chloride and benzethonium chloride. In general, the formulations of the peptides or antigen constructs may include components in amounts that will not adversely affect the production of stable forms, and in amounts suitable for effective, safe pharmaceutical administration. For example, other pharmaceutically acceptable excipients well-known to those skilled in the art may form part of the vaccines or formulations according to the invention. For example, these include salts, various fillers, additional buffer agents, chelating agents, antioxidants, co-solvents and the like.

In a preferred embodiment of the invention the immunogenic construct is associated with a liposomal formulation. For example, this can be effected in such a

way that the immunogenic construct is entrapped in a liposome or anchored on the liposome surface. It is well-known to those skilled in the art that artificial or natural membranes of liposomes may have an immune-stimulating effect, especially in those cases where the antigenic components are coupled to the surface of liposomes or entrapped inside the liposomes or simply mixed together with the liposomes. In a preferred fashion the immune-stimulating effect is increased by spiking the liposomes with transmembranous or fusogenic glycoproteins. For example, such formulations of liposomes can be applied on the parenteral route. Using well-known methods, e.g. a spray, such formulations can be applied nasally on the mucosa of the nasal cavity. In a preferred fashion, a mucosal immune response that can be induced by the spray is suitable in the treatment of SARS. Especially in nasal administration, the antigenic component or the immunogenic construct must be applied on the mucosa in a state permitting penetration of the mucosa or absorption thereby. For this reason, the vesicle must be biocompatible with the mucus and have a certain degree of hydrophilicity. For example, such structures are known to those skilled in the art from EP 0 682 528, the teaching of which is hereby incorporated in the disclosure of the invention. The liposomal composition may comprise one or more additional pharmaceutical carriers selected from surface-active substances and absorption-promoting agents such as polyoxyethylene alcohol ethers, bile salts and derivatives thereof, fusidinic acid and derivatives thereof, oleic acid, lecithin, lysolecithins, Tween® 21 to 85, etc., water-absorbing polymers such as glycofurol, polyethylene glycol 200 to 7500, polyvinylpyrrolidone, propylene glycol or polyacrylic acid, gelatin, cellulose and derivatives etc.; substances inhibiting enzymatic degradation, such as aprotinin etc.; organic solvents such as alcohols, e.g. ethanol, glycerol, benzyl alcohol etc.; or ethyl acetate etc.; hydrophobic agents such as vegetable oil, soybean oil, peanut oil, coconut oil, corn oil, olive oil, sunflower oil, "miglyols" or mixtures thereof, etc.; pH regulators such as nitric acid, phosphoric acid, acetic acid, citrates, etc.; preservatives and agents regulating the osmotic pressure, such as glycerol, sodium chloride, methyl para-oxybenzoate, benzoic acid, etc.; liposomes and/or emulsion formulations such as lecithins etc.; micro-encapsulated formulations; propellants such as butane.

In another embodiment of the invention the peptide sections are optionally associated with each other or, coupled to a carrier, enclosed in liposomes, and such enclosure in liposomes does not necessarily imply - in the meaning of the invention - that the peptides are present inside the liposomes. Enclosure in the meaning of the invention may also imply that the peptides are associated with the membrane of the liposomes, e.g. in such a way that the peptides are anchored on the exterior membrane. Such a representation of the inventive peptides in or on liposomes is advantageous in those cases where a person skilled in the art selects the liposomes such that the latter have an immune-stimulating effect. Various ways of modifying the immune-stimulating effect of liposomes are known to those skilled in the art from DE 198 51 282. The lipids can be ordinary lipids, such as esters and amides, or complex lipids, e.g. glycolipids such as cerebrosides or gangliosides, sphingolipids or phospholipids.

In a preferred embodiment of the invention the first helical structure is a C-terminal helix and the second α-helical structure is an N-terminal helix of the viral envelope protein. For example, when selecting gp41 of HIV to generate an immunogenic construct, a person skilled in the art can preferably select a region between the fusion domain and the N-terminal helix and a region between the transmembrane passage and the C-terminal helical structure; here, the fusion domain, the region of the transmembrane passage and/or the α-helical structure, provided it is one, or the α-helical structures can be involved completely or partially in the immunogenic construct. When obtaining the peptides of the invention from HI viruses, these will preferably be peptides or recombinant proteins or DNA corresponding to the amino acids 519 to 564 (N-terminal sequence) and 650 to 683 (C-terminal sequence) of the HIV-1 reference genome (NCBI data base: K03455, HIV HXB2 CG) or fragments or subunits thereof which are functionally analogous to the above-mentioned domains, i.e., capable of inducing neutralizing antibodies and, in a particularly preferred fashion, producing protection against infections. Of course, those skilled in the art will be familiar with the fact that, due to the variability in different subtypes of HIV-1, sequence variations will be present within these sequences, and such sequence variations are also

encompassed by the invention. This also applies to partial sequences of the consensus sequences 519 to 564 and 650 to 683, including chemical modifications of single amino acids, linkage of sequences by means of linkers, and sequences with additionally added amino acids for the purpose of sequence multimerization.

Preferred N-terminal sequences are:

FLGFLGAAGSTMGARSMTLTVQARQLLSGIVQQQNNLLRAIEAQQ
FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLL
FLGAAGSTMGAASVTLTVQARLLLSGIVQQQNNLLRAIEAQQHML
FLGFLGAAGSTMGAASITLTVQARQLLS
FLGFLGAAGSTMGAASMTLTVQARQLLS
FLGFLGAAGSTMGAASLTLTVQARQLLS
LLGFLGAAGSTMGAASITLTVQARQLLS
FLGFLGAAGSTMGAASITLTVQVRQLLS
FLGVLSAAGSTMGAAATALTVQTHTLMK

Preferred C-terminal sequences are:

SQNQQEKNEQELLELDKWAGLWSWFSITNWLWY
SQNQQEKNEQELLELDKWASLWNWFNITNWLWY
SQTQQEKNEQELLELDKWASLWNWFDITNWLWY
NEQDLLALDKWASLWNWFDITNWLWYIK
NEQDLLALDKWANLWNWFDISNWLWYIK
NEQDLLALDKWANLWNWFDITNWLWYIR
NEQELLELDKWASLWNWFDITNWLWYIK
NEKDLLALDSWQNLWNWFDITNWLWYIK
NEQELLELDKWASLWNWFSITQWLWYIK
NEQELLALDKWASLWNWFDISNWLWYIK
NEQDLLALDKWDNLWSWFSITNWLWYIK
NEQDLLALDKWASLWNWFDITKWLWYIK

NEQDLLALDKWASLWNWFSITNWLWYIK
NEKKLLELDEWASIWNWLDITKWLWYIK

Abbreviations: single-letter code for amino acids:

A Alanine
C Cysteine
D Aspartic acid
E Glutamic acid
F Phenylalanine
G Glycine
H Histidine
I Isoleucine
K Lysine
L Leucine
M Methionine
N Asparagine
P Proline
Q Glutamine
R Arginine
S Serine
T Threonine
V Valine
W Tryptophan
Y Tyrosine

According to the invention, a peptide is selected from each group of N-terminal sequences and C-terminal sequences, and the immunogenic construct of the invention can be provided by combining at least two sequences. It can also be applied as a recombinant protein or DNA (vaccine, inoculant).

In another preferred embodiment of the invention the inoculant (vaccine) is a fragment of envelope proteins selected from the group comprising GP2, gp20, gp21, gp30, gp36, gp37, gp40, gp41, gp45, gp160, p15E, E2, HA2 and/or F2. The assignment of the above to individual viruses is illustrated below.

In a preferred embodiment of the invention said at least two peptide sections, recombinant proteins or corresponding DNAs are selected from the group comprising (with N representing N-terminal sequences and C representing C-terminal sequences):

N: AVGLAIFLLVLAIMAITSSLVAATTLVNQHTTAKV
C: SLSDTQDTFGLETSIFDHLVQLFDWTSWKDWIK,
preferably in BIV (transmembranous envelope protein gp40);
N: GVGLVIMLVIMAIVAAAGASLGVANAIQQSYTKAAVQTLAN
C: AMTQLAEEQARRIPEVWESLKDVFDWSGWFSWLKYI,
preferably in CAEV (transmembranous envelope protein);
N: FGISAIVAAIVAATAIARSATMSYVALTEVNKIMEVQNH
C: LAQSMITFNTPDSIAQFGKDLWSHIGNWIPGLGASIIKY,
preferably in EIAV1 (transmembranous envelope protein gp45);
N: SSSYSGTKMACPSNRGILRNWYNPVAGLRQSLEQYQVVKQPDYLLVPE
C: MDIEQNNVQGKIGIQQLQKWEDWVRWIGNIPQYLK,
preferably in FIV (transmembranous envelope protein gp36);
N: GIGLVIVLAIMAIIAAAGAGLGVANAVQQSSYTRTAVQSLANATAAQQN
C: QVQIAQRDAQRIPDVWKALQEAFDWSGWFSWLKYIPW,
preferably in OMVV (transmembranous envelope protein gp41);
N: LGFLGFLATAGSAMGAASLVTAQSRTLLAVIVQQQQQLLDVV
C: EEAQIQQEKNMYELWKLNWWDVFGNWFDLTSWDLTSWIKY,
preferably in SIVmac (transmembranous envelope protein gp41);
N: LGALGFLGAAGSTMGAAAVTLTVQARQLLSGIVQQQNNLL
C: EEAQSQQEKNERDLLELDQWASLWNWFDITKWLWYIK,
preferably in SIVcpz (transmembranous protein gp41);
N: FLGFLGAAGSTMGARSMTLTVQARQLLSGIVQQQNNLLRAIEAQQ

FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLL

FLGAAGSTMGAASVTLTVQARLLLSGIVQQQNNLLRAIEAQQHML

C: SQNQQEKNEQELLELDKWAGLWSWFSITNWLWY

SQNQQEKNEQELLELDKWASLWNWFNITNWLWY

SQTQQEKNEQELLELDKWASLWNWFDITNWLWY

preferably in HIV-1 (transmembranous envelope protein gp41);

N: GIGLVIVLAIMAIIAAAGAGLGVANAVQQSYTRTAVGSLANATAAQQE

C: EAALQVHIAQRDARRIPDAWKAIQEAFNNWSSWFSWLKY,

preferably in Visna virus (transmembranous envelope protein gp41);

N: LGFLGFLATAGSAMGARSLTLSAQSRTLLAGIVQQQQQLL

C: EEAQIQEKNMYELQKLNSWDILGNWFDLISWVKYIQ,

preferably in HIV-2 (transmembranous envelope protein gp36);

N: WGPTARIFASILAPGVAAAQALREIERLACWSVKQANLTTSLL

C: KFQLMKKHVNKIGVDSDPIGSWLRGIFGGIGEWAVH,

preferably in RSV (transmembranous envelope protein gp37);

N: SVSHLSSDCNDEVQLWSVTARIFASFFAOGVAAQALKEIERLA

C: ALQAMKEHTEKIRVEDDQIGDWFTRTFGGLGGWLAK,

preferably in ALV (transmembranous envelope protein gp37);

N: GLSLIILGIVSLITLIATAVTACCSLAQSIQAAHTVDLSSQNVTKVMGT

C: IENSPKATLNIADTVDNFLQNLFSNFPSLHSLNKTL,

preferably in JSRV (transmembranous envelope protein gp36);

N: AVTLIPLLVGLGVSTAVATGTAGLGVAVQSYTKLSHQLINDVQALSSTI

C: KIKNLQEDLEKRRKALADNLFLTGLNGLLPYLLP,

preferably in SMRV (transmembranous envelope protein gp20);

N: AIQFIPLVIGLGITTAVSTGTAGLGVSLTWYTKLSHQLISDBQAISSTI

C: KIKNLQDDLEKRRKQLIDNPFWTGFHLLPYVMPL,

preferably in SRV (transmembranous envelope protein gp20);

N: AVSLTLAVLLGLGITAGIGGSTALIKGPIDLQQGLTSLQIAIDAD

C: SMKKLKEKLDKRQLERQDSQNWYEGWFNNWPWFTT,

preferably in GALV (transmembranous envelope protein p15E);

N: EPVSLTLALLLGGLTMGGIAGVGTGTTALVATQQFQQLQAAMHD

C: SMAKLRERLSQRQKLFESQQGWFEGLFNKSPWFTT,

preferably in MuLV (transmembranous envelope protein p15E);

N: KALLEAQFRLQLQMQMHTDIQALEESISALEKSL

C: NMAKLRERLKQRQQLFDSQQGWFEGWFNRSPWFTT,

preferably in FeLV (transmembranous envelope protein p15E);

N: TAALITGPQQLEKGLSNLHRIVTEDLQALEKSVSNL

C: DHSGAIRDSMSKLRERLERRRREREADQGWFEGWFNRS

preferably in PERV (transmembranous envelope protein p15E);

N: TALIKGPIDLQQGLTSLQIAMDTDLRALQDSISKLED

C: SMRRLKERLDKRQLEHQKNLSWYEGWFNRSPWLTT

preferably in KoRV (transmembranous envelope protein p15E);

N: SPVAALTLGLALSVGLTGINVAVSALSHQRLTSLIHVLEQDQQ

C: PLSQRVSTDWQWPWNWDLGLTAWVRET,

preferably in BLV (transmembranous envelope protein of gp30);

N: AVPVAWLVSALAMGAGVAGGITGSMSLASGKSLLHEV

C: PILQERPPLENRVLTGWGLNWDLGLSQWAREALQ,

preferably in HTLV-1 (transmembranous envelope protein gp21);

N: AVPIAVWSVSALAAGTGIAGGVTGSLSLASSKSLLLEVD

C: SVLQERPPLEKRVITGWGLNWDLGLSQWAREALQ,

preferably in HTLV-2 (transmembranous envelope protein gp30);

N: FPNINENTAYSGENENDCDAELRIWSVQEDDLAAGLSWIPFFGPGI

C: KNISEQIDQIKKDEQKIGRGWGLGGKWWTSDWG,

preferably in Marburg virus (transmembranous glycoprotein gp36);

N: LITGGRRTRREAIVNAQPKCNPNLHYWTQDEGAAIGLAWIPYFGPAA

C: KNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWI,

preferably in Ebola (transmembranous protein GP2);

N: LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF

C: DRLNEVAKNLNESLIDLQELKYEQYEKWPWYVW,

preferably in SARS virus (E2, transmembranous glycoprotein gp36);

N: GLFGAIAGFIENGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAA

C: HDVYRDEALNNRFQIKGVELKSGYKDWILISFA,

preferably in influenza virus (hemagglutinin-2, HA2);

N: FAGVVLAGAALGVATAAQITAGIALHQSMLSSQAIDNLRASLETT

C: IAKLEDAKELLESSKQILRSMKGLSSTSIVY,
preferably in measles virus (fusion protein F2);
N: FAGIAIGIAALGVATAAQVTAAVSLVQAQTNARAAAMKNSIQTNRA
C: TELSKVNASLQNAVKQIKESNHQLQSVSVSSK,
preferably in mumps virus (fusion glycoprotein F2);
N: FFGAVIGTIALGVATAAQITAGIALAEAREARKDIALIKDSIVKTH
C: TNFLEESKTELMKARAIISVGGWHNTESTQ,
preferably in HPV-1 (F2 glycoprotein),

the viruses being abbreviated as follows:

BIV (bovine immunodeficiency virus),
CAEV (caprine arthritis encephalitis virus),
EIAV1 (equine infectious anemia virus),
FIV (feline immunodeficiency virus),
OMVV (ovine Maedi-Visna virus),
SIVmac (simian immunodeficiency virus from macaques),
SIVcpz (simian immunodeficiency virus from chimpanzees),
HIV-1 (human immunodeficiency virus type 1),
HIV-2 (human immunodeficiency virus type 2),
RSV (Rous sarcoma virus),
ALV (avian leukosis virus),
JSRV (Jaagsiekte sheep retrovirus),
SMRV (squirrel monkey retrovirus),
SRV (simian retrovirus),
GALV (gibbon leukemia virus),
MuLV (murine leukemia virus),
FeLV (feline leukemia virus),
KoRV (koala retrovirus)
PERV (porcine endogenous retrovirus)
BLV (bovine leukemia virus),
HTLV-1 (human T cell leukemia virus type 1),

HTLV-2 (human T cell leukemia virus type 2),
SARS (pathogen of severe acute respiratory syndrome), and
HPV-1 (human parainfluenza virus).

By virtue of the disclosure of the inventive at least two peptides, protein domains and corresponding DNAs and their capability of, firstly, binding specific antibodies at least in the event of said peptides and protein domains and, secondly, inducing same, various ways of generating further peptides become apparent to those skilled in the art. The disclosure of the teaching according to the invention enables a person skilled in the art to generate further equivalent peptides functionally analogous to peptides with the sequence:

FLGFLGAAGSTMGAASITLTVQARQLLS,
FLGFLGAAGSTMGAASMTLTVQARQLLS,
FLGFLGAAGSTMGAASLTLTVQARQLLS,
LLGFLGAAGSTMGAASITLTVQARQLLS,
FLGFLGAAGSTMGAASITLTVQVRQLLS,
FLGVLSAAGSTMGAAATALTVQTHTLMK,
FLGFLGAAGSTMGARSMTLTVQARQLLSGIVQQQNNLLRAIEAQQ
FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLL
FLGAAGSTMGAASVTLTVQARLLLSGIVQQQNNLLRAIEAQQHML
AVGLAIFLLVLAIMAITSSLVAATTLVNQHTTAKV,
GVGLVIMLVIMAIVAAAGASLGVANAIQQSYTKAAVQTLAN,
FGISAIVAAIVAATAIARSATMSYVALTEVNKIMEVQNH,
SSSYSGTKMACPSNRGILRNWYNPVAGLRQSLEQYQVVKQPDYLLVPE,
GIGLVIVLAIMAIIAAAGAGLGVANAVQQSSYTRTAVQSLANATAAQQN,
LGFLGFLATAGSAMGAASLVTAQSRTLLAVIVQQQQQLLDVV,
LGALGFLGAAGSTMGAAAVTLTVQARQLLSGIVQQQNNLL,
GIGLVIVLAIMAIIAAAGAGLGVANAVQQSYTRTAVGSLANATAAQQE,
LGFLGFLATAGSAMGARSLTLSAQSRTLLAGIVQQQQQLL,
WGPTARIFASILAPGVAAAQALREIERLACWSVKQANLTTSLL,
SVSHLSSDCNDEVQLWSVTARIFASFFAOGVAAQALKEIERLA,

GLSLIILGIVSLITLIATAVTACCSLAQSIQAAHTVDLSSQNVTKVMGT,
AVTLIPLLVGLGVSTAVATGTAGLGVAVQSYTKLSHQLINDVQALSSTI,
AIQFIPLVIGLGITTAVSTGTAGLGVSLTWYTKLSHQLISDBQAISSTI,
DPVSLTVALLLGGLTMGSLAAGIGTGTAALIETNQFKQLQ,
AVSLTLAVLLGLGITAGIGGSTALIKGPIDLQQGLTSLQIAIDAD,
EPVSLTLALLLGGLTMGGIAGVGTGTTALVATQQFQQLQAAMHD,
EPISLTVALMLGLTVGGIAAGCGTGTKALLEAQFLQLQMQMHTD,
SPVAALTLGLALSVGLTGINVAVSALSHQRLTSLIHVLEQDQQ,
AVPVAWLVSALAMGAGVAGGITGSMSLASGKSLLHEV,
AVPIAVWSVSALAAGTGIAGGVTGSLSLASSKSLLLEVD,
FPNINENTAYSGENENDCDAELRIWSVQEDDLAAGLSWIPFFGPGI,
LITGGRRTRREAIVNAQPKCNPNLHYWTQDEGAAIGLAWIPYFGPAA,
LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDF,
GLFGAIAGFIENGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAA,
FAGVVLAGAALGVATAAQITAGIALHQSMLSSQAIDNLRASLETT,
FAGIAIGIAALGVATAAQVTAAVSLVQAQTNARAAAMKNSIQTNRA,
FFGAVIGTIALGVATAAQITAGIALAEAREARKDIALIKDSIVKTH,
the above preferably being N-terminal sequences, and

NEQDLLALDKWASLWNWFDITNWLWYIK,
NEQDLLALDKWANLWNWFDISNWLWYIK,
NEQDLLALDKWANLWNWFDITNWLWYIR,
NEQELLELDKWASLWNWFDITNWLWYIK,
NEKDLLALDSWQNLWNWFDITNWLWYIK,
NEQELLELDKWASLWNWFSITQWLWYIK,
NEQELLALDKWASLWNWFDISNWLWYIK,
NEQDLLALDKWDNLWSWFSITNWLWYIK,
NEQDLLALDKWASLWNWFDITKWLWYIK,
NEQDLLALDKWASLWNWFSITNWLWYIK,
NEKKLLELDEWASIWNWLDITKWLWYIK,
SQNQQEKNEQELLELDKWAGLWSWFSITNWLWY
SQNQQEKNEQELLELDKWASLWNWFNITNWLWY

SQTQQEKNEQELLELDKWASLWNWFDITNWLWY
SLSDTQDTFGLETSIFDHLVQLFDWTSWKDWIK,
AMTQLAEEQARRIPEVWESLKDVFDWSGWFSWLKYI,
LAQSMITFNTPDSIAQFGKDLWSHIGNWIPGLGASIIKY,
MDIEQNNVQGKIGIQQLQKWEDWVRWIGNIPQYLK,
QVQIAQRDAQRIPDVWKALQEAFDWSGWFSWLKYIPW,
EEAQIQQEKNMYELWKLNWWDVFGNWFDLTSWDLTSWIKY,
EEAQSQQEKNERDLLELDQWASLWNWFDITKWLWYIK,
EAALQVHIAQRDARRIPDAWKAIQEAFNNWSSWFSWLKY,
EEAQIQEKNMYELQKLNSWDILGNWFDLISWVKYIQ,
KFQLMKKHVNKIGVDSDPIGSWLRGIFGGIGEWAVH,
ALQAMKEHTEKIRVEDDQIGDWFTRTFGGLGGWLAK,
IENSPKATLNIADTVDNFLQNLFSNFPSLHSLNKTL,
KIKNLQEDLEKRRKALADNLFLTGLNGLLPYLLP,
KIKNLQDDLEKRRKQLIDNPFWTGFHLLPYVMPL,
SMAKLRERFKQRQKLFESQQGQFEGWYNKSPWETT,
SMKKLKEKLDKRQLERQDSQNWYEGWFNNWPWFTT,
SMAKLRERLSQRQKLFESQQGWFEGLFNKSPWFTT,
NMAKLRERLKQRQQLFDSQQGWFEGWFNRSPWFTT,
PLSQRVSTDWQWPWNWDLGLTAWVRET,
PILQERPPLENRVLTGWGLNWDLGLSQWAREALQ,
SVLQERPPLEKRVITGWGLNWDLGLSQWAREALQ,
KNISEQIDQIKKDEQKIGRGWGLGGKWWTSDWG,
KNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWI,
DRLNEVAKNLNESLIDLQELKYEQYEKWPWYVW,
HDVYRDEALNNRFQIKGVELKSGYKDWILISFA,
IAKLEDAKELLESSKQILRSMKGLSSTSIVY,
TELSKVNASLQNAVKQIKESNHQLQSVSVSSK,
TNFLEESKTELMKARAIISVGGWHNTESTQ,
and the latter preferably being C-terminal sequences.

For example, it is possible to replace single amino acids or groups of amino acids without adversely affecting the activity of the peptides with respect to accomplishing the object of the present invention. For replacement of such amino acids, reference is made to appropriate standard textbooks of biochemistry and genetics.

Various ways of preparing peptides have been disclosed in the prior art. Peptides designed starting from the peptides of the invention using such methods are included in the teaching according to the invention. For example, one way of generating functionally analogous peptides has been described in PNAS USA 1998, Oct. 13, 9521, 12179-84; WO 99/6293 and/or WO 02/38592, and the above teachings are hereby incorporated in the disclosure of the invention. That is, all peptides, peptide fragments or structures comprising peptides generated using the methods mentioned above - starting from the peptides of the invention - are peptides in the meaning of the invention, provided they accomplish the object of the invention, particularly of generating neutralizing antibodies.

As is well-known to those skilled in the art, some amino acids have analogous physicochemical properties so that these amino acids advantageously can be replaced by each other. For example, these include the group of amino acids (a) glycine, alanine, valine, leucine and/or isoleucine; or the amino acids (b) serine and threonine, the amino acids (c) asparagine and glutamine, the amino acids (d) aspartic acid and glutamic acid; the amino acids (e) lysine and arginine, as well as the group of aromatic amino acids (f) phenylalanine, tyrosine and/or tryptophan. Amino acids within one and the same group (a-f) can be replaced with one another. Furthermore, the amino acids can be replaced by modified amino acids or specific enantiomers. Further modifications are possible in accordance with the teaching of WO 99/62933 or WO 02/38592.

In another preferred embodiment the peptide comprises a linker and/or a spacer selected from the group comprising α-aminocarboxylic acids as well as homo- and heterooligomers thereof, α,ω-aminocarboxylic acids and branched homo- or

heterooligomers thereof, other amino acids, as well as linear and branched homo- or heterooligomers (peptides); amino-oligoalkoxyalkylamines; maleinimidocarboxylic acid derivatives; oligomers of alkylamines; 4-alkylphenyl derivatives; 4-oligoalkoxyphenyl or 4-oligoalkoxyphenoxy derivatives; 4-oligoalkylmercaptophenyl or 4-oligoalkylmercaptophenoxy derivatives; 4-oligoalkylaminophenyl or 4-oligoalkylaminophenoxy derivatives; (oligoalkylbenzyl)phenyl or 4-(oligoalkylbenzyl)phenoxy derivatives, as well as 4-(oligoalkoxybenzyl)phenyl or 4-(oligoalkoxybenzyl)phenoxy derivatives; trityl derivatives; benzyloxyaryl or benzyloxyalkyl derivatives; xanthen-3-yloxyalkyl derivatives; (4-alkylphenyl)- or ω-(4-alkylphenoxy)alkanoic acid derivatives; oligoalkylphenoxyalkyl or oligoalkoxyphenoxyalkyl derivatives; carbamate derivatives; amines; trialkylsilyl or dialkylalkoxysilyl derivatives; alkyl or aryl derivatives and/or combinations thereof; other possible structures have been described in EP 1 214 350 which hereby is incorporated in the disclosure of the invention.

In a preferred fashion, synthetic peptides corresponding to the N-terminal and/or C-terminal sequences or being fragments thereof can be multimerized by chemical crosslinkers or coupled to a carrier molecule such as BSA, dextran, KLH or others. Chemical crosslinkers used to this end are listed in "Bioconjugate Techniques", Greg T. Hermanson, Academic Press, 1996, which hereby is incorporated in the disclosure of the teaching according to the invention. Preferred crosslinkers are homobifunctional crosslinkers, preferably NHS esters such as DSP, DTSSP, DSS, BS, DST, sulfo-DST, BSOCOES, sulfo-BSOCOES, EGS, sulfo-EGS, DSG or DSC, homobifunctional imidoesters such as DMA, DMP, DMS or DTBP, homobifunctional sulfhydryl-reactive crosslinkers such as DPDPB, BMH or BMOE, difluorobenzene derivatives such as DFDNB or DFDNPS, homobifunctional photoreactive crosslinkers such as BASED, homobifunctional aldehydes such as formaldehyde or glutaraldehyde, bisepoxides such as 1,4-butanediol diglycidyl ethers, homobifunctional hydrazides such as adipic dihydrazides or carbohydrazides, bisdiazonium derivatives such as bisdiazotized o-tolidine, benzidine or bisalkylhaloid.

Also preferred are heterobifunctional crosslinkers, especially amine-reactive and sulfhydryl-reactive crosslinkers such as SPDP, LC-SPDP, sulfo-LC-SPDP, SMPT, sulfo-LC-SMPT, SMCC, sulfo-SMCC, MBS, sulfo-MBS, SIAB, sulfo-SIAB, SMPB, sulfo-SMBP, GMBS, sulfo-GMBS, SIAX, SIAXX, SIAC, SIACX or NPIA, carbonyl-reactive and sulfhydryl-reactive crosslinkers such as MPBH, M_2C_2H or PDPH, amine-reactive and photoreactive crosslinkers such as NHS-ASA, sulfo-NHS-ASA, sulfo-NHS-LC-ASA, SASD, HSAB, sulfo-HSAB, SANPAH, sulfo-SANPAH, ANB-NOS, SAND, SADP, sulfo-SADP, sulfo-SAPB, SAED, sulfo-SAMCA, p-nitrophenyldiazopyruvate or PNP-DTP, sulfhydryl- and photoreactive crosslinkers such as ASIB, APDP, benzophenone-4-iodoacetamide or benzophenone-4-maleinimide, carbonyl-reactive and photoreactive crosslinkers such as ABH, carboxylate-reactive and photoreactive crosslinkers such as ASBA, arginine-reactive crosslinkers such as APG, trifunctional crosslinkers such as 4-azido-2-nitrophenylbiocytin 4-nitrophenyl ester, sulfo-SEBD, TSAT and/or TMEA.

In another preferred embodiment of the invention the peptides of the invention and structures produced in a recombinant fashion are linked by peptide bridges having a length of from 0 to 50 amino acids. Also included are recombinant proteins consisting of two N-terminal and one C-terminal sequence, or hexamers consisting of three N-terminal sequences and three C-terminal sequences, or multimers of the above-mentioned recombinant structures, wherein a peptide bridge of 0 to 50 amino acids can be present between each of the N- and C-terminal sequences. For purification, solubilization, or changes in conformation, the peptides can be provided with specific fusion components either on the N or C terminus, such as CBP (calmodulin binding protein), His-tag and/or others. Similar constructs can also be encoded by DNA used in immunization.

According to another particularly preferred embodiment of the invention, the peptide mixture and protein mixture are selected from the group comprising:

a) at least two peptides or recombinant proteins comprising an amino acid sequence in accordance with SEQ ID Nos. 1 to 104,

b) peptides or recombinant proteins comprising an amino acid sequence which has sufficient homology to be functionally analogous to an amino acid sequence in accordance with a),

c) peptides or recombinant proteins in accordance with an amino acid sequence a) or b) modified by deletions, additions, substitutions, translocations, inversions and/or insertions and functionally analogous to an amino acid sequence in accordance with a) or b).

In one particular embodiment of the invention the amino acid sequence, e.g. the peptide or recombinant protein having sufficient homology to be functionally analogous to one of the amino acid sequences in accordance with SEQ ID Nos. 1 to 104, has at least 40% homology thereto.

In another preferred embodiment the above amino acid sequences have at least 60%, preferably 70%, more preferably 80%, and especially preferably 90% homology to one of the amino acid sequences in accordance with SEQ ID Nos. 1 to 104.

In a particularly preferred embodiment of the invention the peptide or recombinant protein is essentially constituted of the amino acid sequence:

a) synthetic peptides E1 (LGAAGSTMGAASVTLTVQARLLLS, FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLL) and E2 (NEQELLELDKWASLWNWFDITNWL or SQNQQEKNEQELLELDKWASLWNWFNITNWLWY)

b) hybrid I (HIV sequences underlined)

LITQARQLLSDIVQQQRIVTEDLQALEKSVSNLEESLTSLSEVVLQNRRGLDLLF LKKEGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRRREELDKWASLWNW FN

c) hybrid II (HIV sequences underlined)

LITGASVTLTVQARQLLSDIVQQQRIVTEDLQALEKSVSNLEESLTSLSEVVLQN RRGLDLLFLKKEGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRRREELDK WASLWNWFNITNWLWY

d) loop I: (HIV sequences underlined)

LGAAGSTMGAASVTLTVQARLLLSSSPSSNEQELLELDKWASLWNWFDITNWL

More specifically, the peptide is constituted of modifications of the above sequences obtained according to WO 99/62933 and WO 02/38592, and these peptides obviously generate and bind antibodies in the meaning of the invention.

In another preferred embodiment of the invention the peptide sections of the immunogenic construct are associated among each other with other peptides or proteins or with a carrier. For example, mutual linkage of said at least two inventive peptides of the immunogenic constructs via peptide or non-peptide bonds can be envisaged. Non-peptide bonds are well-known to those skilled in the art, comprising e.g. imino, ester, azo, hydrazide, semicarbazide and other bonds. The carriers in the meaning of the invention can be proteins which, owing to their immunogenic properties, stimulate an antibody response, but also pharmaceutical vehicles well-known to those skilled in the art, such as QS-21, GPI-0100 or other saponines, water-oil emulsions such as Montanides, polylysine, polyarginine compounds, or others, e.g. phosphate-buffered saline, water, various kinds of detergents, sterile solutions and the like.

The invention also comprises antibodies produced or induced by the peptides of the invention or by the immunogenic construct of the invention. The peptides or

proteins can be produced by chemical means or by using recombinant DNA technology or in another way. In the production of the antibodies according to the invention, at least one immunogenic construct of the invention is contacted with an organism in such a way that the latter is capable of producing antibodies against said construct, which can be obtained and isolated by a person skilled in the art, using well-known methods.

However, the invention also relates to anti-idiotype antibodies. Antibodies bear idiotypes, i.e., regions near their antigen recognition site, which are antigenic themselves and capable of stimulating antibody production. Antibodies specific to the antigen binding site are referred to as paratope-specific anti-idiotype antibodies. These antibodies bear the same recognition site as the antigen initially having stimulated the antibody production; Marx, "Making Antibodies without Antigens", 1986. Thus, in the meaning of the invention, paratope-specific anti-idiotype antibodies, partly having the same structure as HIV, SARS or other viruses, can be produced by immunizing an animal with monoclonal HIV-1, SARS or FeLV antibodies to the viruses mentioned above. The paratope-specific anti-idiotype antibodies, which bear a particular structure identical to that of the immunogenic portions of the above-mentioned viruses, are suitable in triggering an immune response and, as a consequence, can also be used as vaccines.

The invention also comprises pharmaceutical agents including at least one of the immunogenic constructs of the invention, optionally together with a pharmaceutically tolerable carrier. A pharmaceutical agent in the meaning of the invention is any agent in the field of medicine, which can be used in prophylaxis, diagnosis, therapy, follow-up or aftercare of patients who have come in contact with enveloped viruses, including retroviruses, in such a way that a pathogenic modification of their overall condition or of the condition of particular regions of the organism could establish at least temporarily. Thus, for example, the pharmaceutical agent in the meaning of the invention can be a vaccine or an immunotherapeutic agent. In addition to the immunogenic construct, the pharmaceutical agent in the meaning of the invention may include e.g. an acceptable salt or components

thereof. For example, these can be salts of inorganic acids such as phosphoric acid or salts of organic acids.

Furthermore, the salts can be free of carboxyl groups and derived from inorganic bases such as sodium, potassium, ammonium, calcium or iron hydroxides, or from organic bases such as isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine and others. Examples of liquid carriers are sterile aqueous solutions including no further materials or active ingredients, e.g. water, or those comprising a buffer such as sodium phosphate with a physiological pH or a physiological salt solution or both, such as phosphate-buffered sodium chloride solution. Other liquid carriers may comprise more than just one buffer salt, e.g. sodium and potassium chlorides, dextrose, propylene glycol or others. Liquid compositions of the pharmaceutical agents may additionally comprise a liquid phase, with water being excluded, however. Examples of such additional liquid phases are glycerol, vegetable oils, organic esters or water-oil emulsions. The pharmaceutical composition or pharmaceutical agent typically includes a content of at least 0.01 wt.-% of peptides according to the invention, relative to the overall pharmaceutical composition. The respective dose or dosage range for administering the pharmaceutical agent according to the invention is sufficiently high in order to achieve the desired prophylactic or therapeutic effect of forming neutralizing antibodies. In this context, the dose should not be selected in such a way that undesirable side effects would dominate. In general, the dose will vary with the patient's age, constitution, sex and, of course, depending on the severity of the disease. The individual dose can be adjusted both with reference to the primary disease and with reference to the occurrence of additional complications. Using well-known means and methods, the exact dose can be determined by a person skilled in the art, e.g. by determining the antibody titer as a function of dosage or as a function of the inoculation regime or pharmaceutical carrier and the like. Depending on the patient, the dose can be selected individually. For example, a dose of pharmaceutical agent just tolerated by a patient can be such that the range thereof in plasma or locally in particular organs is from 0.1 to 10,000 μM, preferably between 1 and 100 μM. Alternatively, the dose can be

calculated relative to the body weight of the patient. In this event, a typical dose of pharmaceutical agent would have to be adjusted e.g. in a range between 0.1 μg and 100 μg per kg body weight, preferably between 1 and 50 μg/kg. Furthermore, however, it is also possible to determine the dose on the basis of particular organs rather than the whole patient. For example, this would be the case when placing the pharmaceutical agent according to the invention, e.g. in a biopolymer incorporated in the respective patient, near specific organs by means of surgery. Several biopolymers capable of liberating peptides or recombinant proteins in a desirable manner are known to those skilled in the art. For example, such a gel may include 1 to 1000 μg of amino acid sequences of the invention, e.g. peptides or recombinant proteins, or of pharmaceutical agent per ml gel composition, preferably between 5 and 500 μg/ml, and more preferably between 10 and 100 mg/ml. In this event, the therapeutic agent is administered as a solid, gel-like or liquid composition.

In a preferred fashion the pharmaceutical agent is employed as a vaccine, following infection, or as a preventive vaccination. Advantageously, vaccination is effected in such a way that, following application, an active vaccine protection is developed in the organism. Of course, it is also possible to effect vaccination immediately prior to or shortly after an infection, or as a therapy with a plurality of applications. Those skilled in the art are familiar with the fact that induction of a response of neutralizing antibodies can be advantageous at virtually any point in time following infection, so that vaccination in the meaning of the invention could also be application of the inventive pharmaceutical agent weeks, months, years or decades after infection with a particular virus.

To support the immune response, the pharmaceutical agent can include further immunogenic components in a preferred embodiment of the invention, particularly those selected from the group consisting of bordetella, hemophilus, borrelia, pseudomonas, corynebacteria, mycobacteria, streptococcus, salmonella, pneumococcus, staphylococcus and/or clostridia. As is well-known, administration of antigens alone will frequently fail to result in sufficient formation of antibodies.

Under certain preconditions and conditions, epitopes may have only weak immunogenicity, and a corresponding immune response, e.g. formation of antibodies, can therefore be increased by increasing the immunogenicity of weakly immunogenic substances. Apart from well-known aluminum compounds, lipid-containing compounds or KLH and others, it is possible in the meaning of the invention to increase the immune response by joint or parallel administration of particular structures from micro-organisms. The mechanism of action of such additionally administered, highly immunogenic structures is not relevant in the context with the teaching of the invention; what is crucial is that there will be a desired immune response. For example, by administering the above-mentioned immunogenic portions of micro-organisms, the immune response can also take place e.g. in such a way that these structures initially activate a so-called innate immune reaction in the body, thereby enabling induction of an antibody response in the meaning of Fearon, 1997, during the further course. Possibly required inactivation of the above-mentioned microbial immunogens can be effected by means of a chemical or physical treatment, e.g. treatment using formaldehyde, heat treatment, UV irradiation or ultrasonic treatment. Of course, the immunogenic component can also be derived from other viruses, preferably those selected from the family of hepadnavirus, herpesvirus, poxvirus, adenovirus, papovavirus, parvovirus, retrovirus, togavirus or flavivirus; for example, the virus can be a HIV, herpes simplex virus, influenza virus, hepatitis virus. The modifications already illustrated for peptides are also applicable to the pharmaceutical agent.

The immunogenic component in this case can be a protein of the above viruses or a fragment, i.e., a peptide thereof. For example, it is possible to combine the immunogenic component in the pharmaceutical agent with said two inventive peptides or recombinant proteins, i.e., the construct of the invention. Such combination in the meaning of the invention is preferably effected by covalent binding. Obviously, it is also possible to adsorb the immunogenic component and the inventive peptides together on a carrier, and the latter can be an albumin, a KLH or another pharmaceutically tolerable carrier, for example.

Accordingly, the invention also comprises a method for the production of an immunogenic construct consisting of (a) the inventive peptides or recombinant proteins or (b) the pharmaceutical agent of the invention method and (c) micro-organisms used as additional immunogens, such as bacteria or viruses. A preferred method comprises the steps of treating the inventive peptides and/or the immunogenic microbial structures with an activator suitable for covalent binding, optionally removing excess activator, incubating the solution thus obtained, and purification thereof. As activators for covalent binding, homo-, hetero-, bifunctional crosslinkers such as N-hydroxysuccinimide esters, imido esters, maleinimido derivatives, N-hydroxysuccinimides, pyridyl disulfides or compounds containing keto groups can preferably be used. Purification can be effected by means of centrifugation, filtration, precipitation, dialysis or chromatographic methods. Preferred as chromatographic methods are gel filtration, purification by affinity chromatography, or ion exchanger chromatography. In those cases where the inventive peptides in the pharmaceutical agent are applied together with an immunogenic micro-organism component on an adsorbent carrier material, the carriers can be metals, insoluble or colloidal metal compounds or polymer compounds, as well as lipid vesicles. Among the metals, gold or platinum or metals such as aluminum or iron are preferred; among the insoluble or colloidal metal compounds, adjuvants such as aluminum, zinc and/or iron hydroxides are preferred, among other things. As to the polymer compounds, any absorbable or biodegradable material can be used. Obviously, non-degradable polymer compounds can also be used, as long as they are physiologically tolerated, e.g. latex. In addition to micro-organisms or components thereof or other viruses as immunogens, it is obviously also possible to use nucleic acids in order to enhance an immune response to the peptides according to the invention.

In another preferred embodiment of the invention the pharmaceutical agents also comprise cytokines, especially interleukin-2 and/or CSF. Substances of this type can be used to enhance the effect, preferably for immune stimulation. They are mixed with the peptides of the invention or with the pharmaceutical agent according to well-known methods and administered in a suitable formulation and

dosage. Formulation, dosage and suitable components are well-known to those skilled in the art.

The invention also relates to the amino acid sequences in accordance with SEQ ID Nos. 1-104, especially for use in medicine. These sequences represent a selection of well-known larger amino acid sequence regions, the above selection furnishing the surprising result of inducing neutralizing antibodies, especially when at least two of the amino acid sequences are used in combination. These amino acid sequences are particularly preferred in the context with a medical indication, i.e., as amino acid sequences to be used for a specified purpose. Of course, the well-known sequence ELDKWA and other sequences disclosed in the above-mentioned references will not be claimed herein. The invention also relates to the neutralizing antibodies produced using the immunogenic construct according to the invention.

The invention also relates to a diagnostic kit comprising a pharmaceutical agent according to the invention. For example, the kit can be used for diagnosis and in monitoring the course and/or therapy of viral diseases, and the following viruses are preferably selected:

BIV (bovine immunodeficiency virus),
CAEV (caprine arthritis encephalitis virus),
EIAV1 (equine infectious anemia virus),
FIV (feline immunodeficiency virus),
OMVV (ovine Maedi-Visna virus),
SIVmac (simian immunodeficiency virus from macaques),
SIVcpz (simian immunodeficiency virus from chimpanzees),
HIV-1 (human immunodeficiency virus type 1),
HIV-2 (human immunodeficiency virus type 2),
RSV (Rous sarcoma virus),
ALV (avian leukosis virus),
JSRV (Jaagsiekte sheep retrovirus),

SMRV (squirrel monkey retrovirus),
SRV (simian retrovirus),
GALV (gibbon leukemia virus),
MuLV (murine leukemia virus),
FeLV(feline leukemia virus),
BLV (bovine leukemia virus),
HTLV-1 (human T cell leukemia virus type 1),
HTLV-2 (human T cell leukemia virus type 2),
SARS (pathogen of severe acute respiratory syndrome)
HPV-1 (human parainfluenza virus),
and other enveloped viruses.

The diagnostic kit is used to detect antiviral antibodies against epitopes on the immunogenic construct, detect neutralizing antibodies against the immunogenic construct and/or a virus.

The kit can optionally include instructions or information as to the pharmaceutical provision or the procedure of therapeutical treatment. For example, the information can be an instruction leaflet or other medium providing the user with information in which therapeutical procedure the above-mentioned substances, i.e., especially the inventive peptides or the inventive pharmaceutical agent, particularly the vaccine, should be used. In particular, the instruction leaflet includes detailed and/or important information about the therapeutical treatment. Obviously, the information need not necessarily be in the form of an instruction leaflet, and the information may also be imparted via the Internet, for example.

On the one hand, the diagnostic kit relates to an immunoassay for the detection of antibodies directed against enveloped viruses, including retroviruses, and, on the other hand, to the detection of the viral exposure (viral antigens) of an organism. To this end, appropriate samples are taken, wherein antibodies or viral antigens are to be detected. A sample in the meaning of the invention refers to a biological or chemical material collected by sampling, or to a portion or small

amount of such material, the composition of which is to be tested by chemical, biological, clinical or similar means, particularly for the presence of neutralizing antibodies.

More specifically, sampling is effected in such a way that the collected partial amount corresponds to an average of the total amount. Of course, it can also be preferred that the collected partial amount does not correspond to the average of a larger amount. The characteristics determined by investigating the sample are used to assess the amount detected through the sample, allowing conclusions as to the overall amount, e.g. blood in an organism or lymph. For investigation, the samples can be pre-treated by mixing, addition of enzymes or markers, or in another way. Various ways of pre-treating samples are known to those skilled in the art. More specifically, samples can be all biological materials such as biological tissues and fluids, e.g. blood, lymph, urine, cerebral fluid and others. As used herein, sample therefore refers to any substance containing or presumably containing neutralizing antibodies and comprises a sample of tissue or fluid isolated from an individual or individuals, including, but not limited to, e.g. skin, plasm, serum, lymph, urine, tears, swabs, tissue samples, organs, tumors, as well as samples of cell culture components. For example, such a sample can be investigated for the presence of binding antibodies as follows:

a) coating a solid phase with the immunogenic construct of the invention,

b) incubating the solid phase with the biological sample,

c) incubating the solid phase with an anti-human antibody capable of detecting the classes IgA, IgM, IgG, which antibody is labelled with a detectable label, and

d) detecting the label in order to determine the presence of binding antibodies against the above-mentioned viruses in the sample.

The sample may include varying concentrations of urea, but may also have no urea (avidity determination).

Detection of neutralizing antibodies in this way is difficult and is not quantitative, so that detection thereof is effected in an infection assay wherein the inhibition of infection of non-infected cells by the respective virus from the list above is detected by the neutralizing antibodies.

By means of competitive analyses using the immunogenic construct, quantitative determination of the amount specific to the epitope on the immunogenic construct is possible from the amount of neutralizing antibodies. A correlation between the amount of the above-mentioned antibodies binding the immunogenic construct and the amount of neutralizing antibodies has been found, and therefore, it is possible to perform a new rapid test wherein, for the first time, a large number of serums can be tested for neutralizing antibodies within a short period of time. This test permits propositions as to the success of immunization in the event of prophylactic application, as well as to the progression of the infection. Also, monitoring the therapeutic success in the event of therapeutic application is possible. Consequently, it is also possible to provide a kit for the detection of neutralizing antibodies against the immunogenic construct on the basis of a neutralization assay using appropriate competitive analyses, as well as a new rapid method for the detection of neutralizing antibodies on the basis of an ELISA, using the complete immunogenic construct.

The diagnostic kit also comprises the detection of a viral antigen to determine the viral exposure in the above-listed samples as follows:

a) coating a solid phase with antibodies against the viral antigens to be found, said antibodies being obtained from an animal or human following immunization with the immunogenic construct of the invention,

b) incubating the solid phase with the biological sample,

c) incubating the solid phase with a second antibody against the viral antigens to be found, said antibody being different from the first one and likewise obtained from an animal or human following immunization with the immunogenic construct of the invention,

d) detecting the coupled second antibody so as to determine the amount of bound antigen.

The invention also relates to an amino acid sequence selected from the group comprising the sequences SEQ ID Nos. 1 to 104 for use in the fields of therapeutic treatment and diagnostic methods in a human or animal body.

Accordingly, the invention relates to peptides, peptide fragments, recombinant proteins or fragments thereof, membrane-associated proteins of particular viruses for specific therapeutic or diagnostic uses. In a preferred fashion, a mixture of at least two peptides or recombinant proteins from the sequence of a viral envelope protein is used in accordance with the selection described according to the invention.

The invention also relates to a method of inducing an antibody response, in which method the immunogenic construct of the invention and/or the pharmaceutical agent of the invention is contacted with an organism, preferably human or animal. The method preferably comprises formulating the neutralizing antibody with a pharmaceutically acceptable carrier. The disclosure of the peptides of the invention enables a person skilled in the art to use them as antigen to induce neutralizing antibodies. Those skilled in the art will be aware of the fact that the peptides of the invention, which obviously can also be used in the form of the pharmaceutical agent of the invention, can be applied at varying dosages to an organism, especially a human patient. Application should be effected in such a way that neutralizing antibodies are generated in an amount as large as possible. Concentration and type of application can be determined by a person skilled in the art using routine tests. The following applications are possible: oral in the

form of powders, tablets, juice, drops, capsules and the like; rectal in the form of suppositories, solutions and the like; parenteral in the form of injections, infusions of solutions, inhalations of vapors, aerosols and powders, as well as local in the form of ointments, pads, dressings, lavages and the like.

In a preferred fashion, contacting with the immunogenic constructs and/or with the pharmaceutical agent can be prophylactic or therapeutic. In prophylactic vaccination, infection with the above-mentioned viruses should be prevented at least in such a way that, following invasion of single viruses, e.g. in a wound, further growth thereof would be massively decreased or invaded viruses virtually completely destroyed. In therapeutic induction of an immune response, an infection is already present in the patient, and induction of the neutralizing antibodies is effected in order to destroy viruses already present in the body or inhibit growth thereof.

The invention also relates to a method of generating an antibody, preferably a neutralizing antibody, against a disease from enveloped viruses, including retroviruses, in which method an organism is contacted with an immunogenic construct, optionally together with an immunogenic component and/or a pharmaceutical agent according to the invention, thereby inducing a humoral immune response by formation of antibodies, and the antibodies are subsequently obtained from the organism. In a preferred fashion the neutralizing antibodies thus obtained can be used in passive immunization.

Also, it is possible to obtain monoclonal antibodies which are used following appropriate humanization, among other things. Moreover, antibody-producing cells can be obtained from inoculated or infected individuals, which cells produce neutralizing antibodies directed against the immunogenic construct of the invention and applied in the form of monoclonal antibodies in passive immunization.

In passive immunization, there is no intrinsic immune reaction or essentially no intrinsic immune reaction to particular viruses in the body of a patient; instead,

the antibodies are introduced into the patient, e.g. in the form of an antiserum. In contrast to active immunization, passive immunization essentially assumes the function of curing as rapidly as possible an infection already having occurred or protecting immediately against infection with viruses. From passive immunization against hepatitis A, hepatitis B and FSME, for example, various inoculation schemes for passive immunization are known to those skilled in the art. Using routine tests, such inoculation schemes can be adapted to specific viruses such as HIV, feline leukemia virus and others. In a preferred fashion the antibodies used in passive immunization can be monoclonal antibodies.

In another preferred embodiment of the invention the inventive immunogenic components or the inventive pharmaceutical agent or the inventive kit are used in the diagnosis, prophylaxis, therapy, follow-up and/or aftercare of retroviral diseases.

Without intending to be limiting, the invention will be explained in more detail with reference to the following example.

Example

1. Materials and methods

1.1 Cloning

The recombinant gp41 constructs were generated on the basis of the HIV-1 molecular clone pNL4-3 (NIH, #114). Using specific primers (Sigma ARK), the coding region for the ectodomain was amplified by means of PCR. The coding regions of the hybrid constructs (referred to as hybrid I and II hereinafter), consisting of a PERV-p15E ectodomain backbone with inserted HIV epitope, were generated using a two-step mutagenesis PCR (27). The coding region for hybrid II is based on that of hybrid I, merely expanded at both flanks by HIV sequences.

The coding regions for constructs consisting of the epitopes of E1 and E2 of gp41 (referred to as loop I hereinafter) from HIV-1 and linked by a short peptide bridge were generated by hybridization of 80mer oligonucleotides (Sigma ARK) and a fill-in reaction using PCR. The PCR product was ligated into the multiple cloning site of the pCal-n expression vector (Stratagene) via a BamHI restriction site at the 5' end and an XhoI restriction site at the 3' end of the coding strand. These constructs were used to transform chemo-competent, codon-optimized *E. coli* BL21 DE3 (Stratagene).

DNA from feline embryonal fibroblasts, FEA, producing FeLV-A, was isolated using a Qiagen DNA Isolation Kit. Using the specific primers, a sequence corresponding to the ectodomain of p15E was amplified by means of PCR, subsequently cloned into the pCal-n vector, and expressed in *E. coli* BL21 DE3. The recombinant p15E, coupled to the 4 kDa calmodulin binding protein (CBP) at the N terminus, was purified using affinity chromatography.

Recombinant CBP fusion proteins

rgp41:

MGCTSMTLTVQARQLLSDIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAV
ERYLKDQQLLGIWGCSGKLICTTAVPWNASWSNK SLEQIWNNMTWMEWDREI
NNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITNWLWYIK

Hybrid I (HIV sequences underlined)

LITQARQLLSDIVQQQRIVTEDLQALEKSVSNLEESLTSLSEVVLQNRRGLDLLF
LKKEGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRRREELDKWASLWNW
FN

Hybrid II (HIV sequences underlined)

LITGASVTLTVQARQLLSDIVQQQRIVTEDLQALEKSVSNLEESLTSLSEVVLQN
RRGLDLLFLKKEGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRRREELDK
WASLWNWFNITNWLWY

Loop I (HIV sequences underlined)
LGAAGSTMGAASVTLTVQARLLLSSSPSSNEQELLELDKWASLWNWFDITNWL

Ectodomain of p15E from FeLV (amino acids 476-583):
LETAQFRQLQMAMHTDIQALEESISALEKSLTSLSEVVLQNRRGLDILFLQEGGL
CAALKEECCFYADHTGLVRDNMAKLRERLKQRQQLFDSQQGWFEGWFNKSP
W

Primers:
rgp41 BamHI fw: cgc gga tcc atg ggc tgc acg tca atg acg ctg

rgp41 XhoI rev: cac ccg ata ctc gag ata cca cag cca att tgt tat g

Hybrid I BamHI fw: cgc cca tcc cta atc aca caa gcg aga cag ctg

Hybrid I XhoI rev: cac ccg ata ctc gag tca gtt gaa cca gtt cca aag

Hybrid I E1mut fw: caa gcg aga cag ctg agt gat att gtt cag caa caa cga att gta acg gaa gat ctc caa gcc c

Hybrid I E1mut rev: ttg ttg ctg aac aat atc act cag cag ctg tct cgc ttg tgt gat tag gga tcc acg cgg

Hybrid I E2mut fw: gaa ctg gat aag tgg gcg tcg ctt tgg aac tgg ttc aac tga gaa ttc aga ctc cag ggg tcg act cga gc

Hybrid I E2mut rev: gtt cca aag cca cgc cca ctt atc cag ttc ttc cct tcg acg cct ctc taa cct ttc tc

Hybrid II BamHI fw: cg gga tcc gga gca tca gta acg ctg acg gta cag cgg aga caa ta ttg tgt gat ata g

Hybrid II XhoI rev: cg ctc gag cta ata cca cag cca att tct tat gtt aaa cca att cca caa act tgc cca tt

Loop I BamHI fw: ggg gat ccc agc ca ttg gag atg tcg aac cag ttc cac aa gaa gcc cat ttg tcc agt tcc agc agt tcc tgt tcg tta gaa gac gga gaa gaa gac a

Loop I XhoI rev: ccg gat ccc tgg gtg ctg ctg gtt cta cca tgg gtg ctg ctt ctg tta ccc tga ccg ttc agg ctc gtc tgc tgc tgt ctt ctt ctc cgt ctt cta acg a

P15E FeLV fw: gcg gat ccc ttg aaa cag ccc agt tca gac aa

p15E FeLV rev: cgg aat tcc cag ggg gac ttg ttg aac cat cc

1.2 Expression of recombinant proteins

For expression, 1 liter of LB-Amp (LB medium with 100 μg/ml ampicillin) heated at 37°C was inoculated with 1 ml of pre-culture and incubated at 37°C and 225 rpm. At an optical density (OD_{600}) of 0.5, the expression cultures were induced for 4 h (37°C and 225 rpm) by adding 1 mM (final concentration) IPTG (Sigma).

1.3 Purification of recombinant proteins

Purification was effected according to the protocol by Stratagene Company for CBP-fused proteins. The purity of the recombinant proteins was examined by means of SDS-PAGE and Western Blot analysis using the monoclonal gp41-specific antibody 2F5 or a serum against p15E from FeLV.

1.4 Synthetic peptides and bioconjugates

Synthetic peptides corresponding to the sequences of the epitopes E1 and E2 of HIV (E1 (LGAAGSTMGAASVTLTVQARLLLS) and E2

(NEQELLELDKWASLWNWFDITNWL) were produced by Jerini Company and purified by means of HPLC. E1 and E2 were used as free antigens (in combination or alone) or on dextran (MW 6 kDa, Sigma). The production of dextran-peptide conjugates was effected either via direct binding of the carboxyl groups included in activated dextran to primary amino groups of the peptides (28), or via a heterobifunctional crosslinker (3-(2-pyridyldithio)propionyl hydrazide, PDPH, Pierce) allowing specific coupling via cysteines attached to the C- or N-terminal end of the peptides (29); overlapping synthetic peptides corresponding to the overall Env (gp120 and gp41) of HIV were obtained from NIH, USA (HIV-Env Peptide Set derived from HIV-1 Isolate MN, Cat#6451). Overlapping and immobilized peptides corresponding to gp41 or p15E of FeLV were produced by Jerine.

1.5 Immunization of rats, goats and mice

Wistar rats (BfR, female, 54-58 days of age), goats, Balb/c mice (female, 10-20 g) were immunized with 500 µg - 1 mg of protein, peptide or peptide conjugate in a mixture of 800 µl PBS/Freund's incomplete adjuvant (1:1), and 200 µl of this was applied i.m. in each hind leg and 400 µl s.c. in the neck. Three animals each time were immunized with the same antigen. The animals were boosted after 4 weeks, using the same injection scheme and the same antigens.

1.6 Serum production

Collection of blood from rats and mice was effected using retrobulbar puncture, and blood of goats was collected from the neck vein. Following storage at 4°C for 20 h, the blood was separated into serum and cellular components by means of centrifugation. The complement factors included in the serum were inactivated by incubation at 56°C for 30 min. The serum was stored at -20°C until used.

1.7 Epitope mapping

Epitope mapping was effected on a Pepspot membrane (Jerini, Berlin), on which peptides overlapping by 11 amino acids, corresponding to the sequences of gp41 and p15E of FeLV, were dotted according to the manufacturer's instructions.

Table 1:

Animals	Number of immunized rats	Antigens used for immunization and boosting	Number of animals with neutralizing serums/number of immunized animals ($\Delta Ct > 2$)
N1-4	4	CBPrgp41	0/4
O4, P1-4, Q1	6	Hybrid II	3/6
Q2-4	3	E1/E2 free peptides	2/3
R1, R2	2	E1/E2-dextran 6 conjugates	1/2
S1-3		E1/E2-PDPH*-dextran 6 conjugates	1/3
S4, T1-2	3	El free peptide	0/3
T3,4, Ul	3	E2 free peptide	0/3
U2-4	3	Hybrid I	2/2
V1-4, W1-2	6	Loop I	n.t.**

* 3-(2-Pyridyldithio)propionyl hydrazide

** Not tested

1.8 ELISA

To determine the change in avidity of mAb 2F5, 50-100 ng of peptides of the N-terminal region of the ectodomain of gp41 from an HIV-Env peptide set arranged in overlapping fashion were applied on Probind ELISA plates (NUNC), each time in combination with Dp178 or peptide P6373. The mAb 2F5 was incubated at 37°C for 2 h at a dilution of 1:25,000 and in the presence of 0-8 M urea. Detection was effected using a polyclonal goat-anti-human serum (1:2000) conjugated to horseradish peroxidase (Sigma). Measurements were made at $OD_{492/560}$. All values were measured in triplicate.

1.9 Neutralization assay

To test the serums for HIV-neutralizing effect, 5×10^4 C8166 cells (human T cell line) in 100 µl of RPMI medium were seeded on 96-well round bottom plates. This was added with 50 µl of HIV-IIIB stock (corresponding to a TCID50 of 2×10^3) and 50 µl of rat serum pre-diluted 1:4 with medium. The plates were incubated for 65 h at 37°C, 95% humidity and 5% CO_2.

The medium supernatant was removed and the cells disrupted by freezing and thawing three times. Interfering proteins were removed from the genomic DNA by addition of proteinase K (Invitrogen) in 100 µl of 1xPCR buffer (Roche) and incubation at 56°C for 3 h. The proteinase K was inactivated by incubation at 95°C for 30 min.

Based on the cell lysate, a real-time PCR was performed with HIV-env-specific primers and an env-specific, Dabcyl-labelled probe (TIB MOLBIOL, Berlin) and PCR Mastermix (Stratagene). The linear serum-specific inhibition was determined using the formula $2^{\Delta ct}$ wherein Δct is the difference of ct(immune serum) and ct(pre-immune serum). Thereafter, the linearized values were converted into percent inhibition. All values were measured in triplicate.

A virus stock of FeLV-A (Glasgow strain) was titrated for non-infected FEA cells, and the titer was $10^{4.76}$ $TCID_{50}$/ml. For the neutralization test, 6,000 FEA cells per well were seeded in a 96-well microtiter plate. Pre-immune and immune serums were heat-inactivated at 56°C for 30 min. 50 µl of virus was added to 1:2 dilutions of serum or purified immunoglobulin, incubated at 37°C for 30 min and added to the cells. Alternatively, the serum concentration was maintained constant at 1:5, and the virus was diluted. After 3 days, the cells were disrupted and lysed (20 mg/ml proteinase K in PCR buffer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.4). The cells were incubated at 56°C for 3 h, and then for 10 more minutes at 95°C to inhibit the activity of proteinase K. Provirus integration was determined quantitatively using PCR.

2. Results

2.1 Epitope mapping of HIV-1-neutralizing monoclonal antibody 2F5

The human monoclonal antibody (mAb) 2F5 shows a wide neutralization spectrum to laboratory strains and other primary isolates. Several teams have demonstrated that the antibody binds to a linear sequence (ELDKWA) within the ectodomain of the transmembranous envelope protein gp41 of HIV-1. This sequence is located a few amino acids from the N terminus of the transmembrane passage. Using an epitope mapping method based on a Pepspot membrane, it was possible to confirm this sequence as a binding site for 2F5 (Fig. 3).

2.2 Binding of the monoclonal antibody 2F5 to Dp178 and CBP-rgp41

As was demonstrated in Western blot analyses, the mAb 2F5 has improved binding to the entire ectodomain of gp41 as compared to the 34mer peptide (Dp178) comprising the epitope ELDKWA described in the literature (Fig. 4).

2.3 ELISA with 2F5 on overlapping peptides of the ectodomain of gp41 in combination with Dp178

To determine whether the complete epitope of 2F5 has an additional portion in the region of the ectodomain of gp41, overlapping 15mers covering the entire ectodomain were applied on ELISA plates in combination with Dp178. 2F5 was incubated on these peptide combinations at varying concentrations of urea (Fig. 5). It is only the peptide P6342 which causes a synergistic augmentation of binding of 2F5 to Dp178. Such enhanced binding at high concentrations of urea when simultaneously applying both domains of the immunogenic construct of the invention is the basis of a rapid test for the detection of neutralizing antibodies by means of an ELISA.

2.4 Binding of 2F5 to P6373 and some peptide 6342-derived peptides with amino acid substitutions

To test to what extent amino acid substitutions in the peptide P6342 would have an influence on enhanced binding of 2F5 to the sequence ELDKWA, the peptide P6373 (NEQELLELDKWASLW) was applied together with modified peptides (P6342 mut 1-5) on ELISA plates and incubated with 2F5 at a concentration of 3 M urea. Fig. 6 shows the evaluation of the ELISA after substraction of the absorption of 2F5 on P6373 alone. It was found that amino acid substitutions in at least 4 positions of peptide P6342 reduces binding of the monoclonal antibody 2F5.

2.5 Stoichiometry of the peptides P6342 and P6373 in mAb 2F5 binding

To examine which stoichiometric ratios would have an influence on the enhanced binding of 2F5 to the peptides P6373 and P6342, varying stoichiometric ratios of P6342 and P6373 were applied in an ELISA and incubated with 2F5 under increasing concentrations of urea (Fig. 7).

It was shown that a 2:1 ratio of P6342 to P6373 results in a significantly enhanced binding of 2F5 to ELDKWA as compared to a ratio of 1:1, implying that two P6342 peptides give better stabilization of ELDKWA than one.

2.6 Inhibition of 2F5-mediated virus neutralization by the peptides E1 and E2

In a neutralization assay, a combination of E1 and E2 (variants of P6342 and P6373 extended to 25mers) has better inhibition of the virus neutralizing effect of 2F5 than E2 alone (Fig. 8A).

Moreover, a stoichiometric ratio of 2:1 (E1/E2) was found to have a stronger inhibition of the neutralizing activity of 2F5 than a ratio of 1:1, similarly as in an ELISA (Fig. 8B).

These data support the ELISA experiments, in which it was shown that the sequence ELDKWA recognized by 2F5 is better stabilized by two N-terminal sequences of the ectodomain of gp41 than by only one, and they also show that this conformation is important to the inhibition of neutralization.

2.7 Immunization tests using recombinant antigens and synthetic peptides

2.7.1 Recovery and characterization of recombinant gp41

The sequence of gp41 amplified on the basis of the NIH-HIV molecular clone pNL4-3 using the specific primers 1 and 2 in the PCR was ligated via the restriction sites of BamHI and XhoI into the vector pCal-n (Stratagene) opened via the same restriction enzymes. The closed vector (pCal-n rgp41) was transformed into BL21 Codon Plus (Stratagene) and expressed therein in an IPTG-dependent fashion. The amino acid sequence of the resulting fusion protein is listed under section 2.1. It was possible to express this sequence as a recombinant fusion protein. However, the fusion protein was insoluble and was therefore used as an aggregate following 8 M urea precipitation to remove soluble bacterial proteins.

2.7.2 Production and characterization of recombinant hybrid constructs constituted of a PERV transmembrane protein backbone with inserted HIV epitopes

Starting from the vector pCal-n rp15E which includes the sequence of the ectodomain of the transmembranous envelope protein p15E from porcine endogenous retrovirus PERV-A (26), sequences of gp41 of HIV-1 were inserted into the PERV-p15E sequence by means of specific primers, thereby eliminating the corresponding domains of p15E (substitution). For this purpose, two different sequences of gp41 were inserted, namely, at the N-terminal end and at the C-terminal end. In addition, the respective substitutes were used with two different lengths (hybrid I and hybrid II). The resulting PCR products were ligated via the restriction sites of BamHI and XhoI into the vector pCal-n (Stratagene) opened via the same restriction enzymes. The closed vector (pCal-n rgp41) was transformed into BL21 Codon Plus (Stratagene) and expressed therein in an IPTG-dependent fashion. The amino acid sequences of the viral portions (p15E and gp41) of the resulting fusion proteins, hybrid I and II, are listed in section 2.1. Similarly, these fusion proteins were insoluble and were therefore used as an aggregate following 8 M urea precipitation to remove soluble bacterial proteins.

2.7.3 Recovery and characterization of recombinant E1-E2 constructs linked via a peptide bridge

Using hybridization of oligonucleotides with subsequent PCR fill-in reaction, the coding sequences for the peptides E1 and E2 linked by the coding region for a peptide bridge five amino acids in length were produced. The resulting PCR product was ligated via the restriction sites of BamHI and XhoI into the vector pCal-n (Stratagene) opened via the same restriction enzymes. The closed vector (pCal-n rgp41) was transformed into BL21 Codon Plus (Stratagene) and expressed therein in an IPTG-dependent fashion. The amino acid sequence of the resulting fusion protein loop 1 is listed in section 2.1. Similarly, these fusion pro-

teins were insoluble and were therefore used as an aggregate following 8 M urea precipitation to remove soluble bacterial proteins.

2.7.4 Immunization of rats and analysis of the immune serums

Rats in groups of at least three animals each were immunized and boosted with the purified recombinant proteins, and also with the synthetic peptides corresponding to the domains of E1 and E2 of gp41 from HIV-1, as described in "Materials and methods". The immune serums were investigated for binding antibodies against gp41 in a Western blot and in an ELISA. The serums were also investigated for neutralizing antibodies in a neutralization assay.

To test the rat serums for virus-neutralizing properties, C8166 in an amount of 5×10^4 was infected with 2×10^3 $TCID_{50}$ HIV-IIIB in the presence of said rat serums. Fig. 9 shows the percent inhibition of virus infection, converted from Δct of the inhibiting effect of pre-immune and boost serums. As positive control, the infection was carried out with 2.5 μg/ml 2F5.

The results of these tests are summarized in Table 1. While all serums reacted with gp41 in ELISA and Western blot, only serums of some animals treated with both peptides E1 and E1 in combination (animals Q2-4), but not alone (animals), of animals treated with the peptides in combination (E1 and E2), directly coupled to dextran 6000 (animals R1, R2), of animals treated with the peptides in combination (E1 and E2), coupled to dextran 6000 via a linker (animals S1-3), and of animals treated with the hybrid I and hybrid II (p15E of PERV with substituted E1 and E2 domains of varying length) (Q4, P2-4, Q1 and U2-3) were virus-neutralizing.

More specifically, the rats O4 and P4, both immunized with hybrid II, and Q3 and S3 immunized with E1 and E2 peptides (free or bound to dextran) show neutral-

izing properties at a dilution of 1:16 that are similarly good as 2.5 μg/ml mAb 2F5.

The recombinant gP41 which, being insoluble, is not really suitable as antigen for immunization, was not capable of inducing neutralizing antibodies (Group N).

As is shown by these results, recent findings in the case of HIV, showing that neutralizing antibodies could be induced following immunization of the recombinant ectodomain of p15E of PERV (30), cannot be utilized. On the one hand, the corresponding antigen was insoluble and, on the other hand, failed to produce any neutralizing antibodies, even when used as insoluble aggregate for immunization. The incapability of inducing neutralizing antibodies cannot be exclusively attributed to insolubility, because neutralizing antibodies were neither produced in a parallel study where solubility had been induced by appropriate fusion proteins (data not shown). In addition, the hybrid II (p15E of PERV with substituted E1 and E2 domains of the longer variant) was neither soluble, but produced neutralizing antibodies.

Legends:

Fig. 1: Spatial structure of gp41 with gp120 on top (Zwick et al. 2001 (5)).

Fig. 2 A): Schematic illustration of the ectodomain of gp41: simplified representation of monomeric gp41, with highlighted fusion peptide at the N terminus, N-terminal helical region (NHR), cysteine-cysteine loop, C-terminal helical region (CHR), and transmembrane passage.

B) Hexameric gp41: The NHRs and CHRs are illustrated in top view. Three N helices are illustrated as trimer consisting of three parallel α-helices with three exterior C helices arranged antiparallel. The interacting amino acids between the helices are shown.

C) Folding mechanism of gp41 during infection. Following binding of CD4 and co-receptor (CCR5/CXCR4), gp210 exposes the underlying gp41 which penetrates the host cell membrane with the fusion peptide. Hydrophobic interactions fold the NHRs and CHRs, pulling the host cell membrane and virus membrane toward each other, which results in fusion of the two membranes.

Fig. 3: Epitope mapping for mAb 2F5 using a Pepspot membrane: 13mer peptides were covalently bound to a nitrocellulose membrane via an acetyl linker. From spot to spot, these peptides overlap by 11 amino acids, covering the entire sequence of gp41. The mAb 2F5 was used at a concentration of 250 ng/ml. The secondary antibody conjugate (anti-human POD) was used at 1:5000. The epitope in the sequence of gp41 is underlined.

Fig. 4: SDS-PAGE (A) and Western blot (B) of Dp178 (lane 1) and recombinantly expressed CBP-rgp41 (lane 2): Although Dp178, which corresponds to the C helix of the ectodomain of gp41, was applied in higher amounts, detection thereof in the Western blot was inferior to that of the recombinant CBP-rgp41 comprising the entire ectodomain of gp41. 2F5 was used in the Western blot at a concentration of 500 ng/ml.

Fig. 5: ELISA investigations on binding of mAb 2F5 to Dp178 in combination with overlapping peptides of the ectodomain of gp41: The NIH peptide set comprises 15mer peptides, each overlapping by 11 amino acids and comprising the entire protein sequence of the envelope protein complex. All peptides were tested in combination with Dp178, but only the results for 10 peptides from the N-terminal region of the ectodomain of gp41 are shown. **(A)** The peptide P6342, but not all of the others, enhanced binding of 2F5 to Dp178. Enhanced binding of 2F5 is synergistic, because mAb 2F5 alone does not recognize P6342, and is still observed with 3 M and 5 M urea. The diagram shows mean values from triplicates. **(B)** Sequences of the peptides employed. DP107 corresponds to the N helix of the ectodomain of gp41 and was co-run as a control.

Fig. 6: Influence of amino acid substitutions in the peptide P6342 on enhanced binding of 2F5 to P6373: Two consecutive amino acids in the sequence of P6342 were replaced by alanine each time. In particular, substitutions in the C-terminal region (P6342 mut4 and mut5) were found to be crucially detrimental to the synergistic augmentation of binding of 2F5 to P6373.

P6342:	AASVTLTVQARLLLS
P6342 mut1:	AAAATLTVQARLLLS
P6342 mut2:	AASVAATVQARLLLS
P6342 mut3:	AASVTLAAQARLLLS
P6342 mut4:	AASVTLTVAARLLLS
P6342 mut5:	AASVTLTVQAAALLS

Fig. 7: ELISA with varying stoichiometric P6342/P6343 combinations at four different concentrations of urea: The ELISA shows that binding of 2F5 to P6342/P6373 is particularly good at a 2:1 ratio of the latter. A larger excess of P6342 does not imply an increase of binding, while a 1:1 ratio results in significantly weaker binding of mAb 2F5 to the two peptides. The diagram shows mean values from triplicates.

Fig. 8: Real-time PCR; (A) Inhibition of the 2F5-dependent virus neutralization by the peptides E1 and E2.
The diagram shows the ct values when using a cell lysate following treatment with varying amounts of E1 (LGAAGSTMGAASVTLTVQARLLLS) and E2 (NEQELLELDKWASLWNWFDITNWL) during infection with HIV. 2F5 was used at a concentration of 2.5 μg/ml.
(B) In neutralization assays as well, a stoichiometric ratio of 2:1 E1/E2 has a stronger inhibiting effect on the virus-neutralizing effect than a 1:1 ratio. All values were measured in triplicate.

Fig. 9: HIV neutralization by rat serums: The columns show the percent inhibition of the HIV-1 IIIB provirus integration in C8166 in the presence of various rat

serums and 2F5, respectively. The rat serums were previously diluted 1:4. 2F5 was used at a concentration of 2.5 μg/ml. The conversion of the ct values into percent inhibition is described under 2.9.

Fig. 10: Recombinant gp41-derived constructs: (A) A recombinant protein including no more than portions of the ectodomain was derived from gp41 of HIV-1. **(B)** A recombinant protein was derived from the transmembranous envelope protein p15E from porcine endogenous retrovirus, and the E1 and E2 domains of gp41 (gray) of HIV 1 were inserted into this protein at topographically identical positions (substitution of the corresponding epitope of p15E). E1 and E2 domains of varying size were selected.

Fig. 11: Recombinant gp41-derived loop protein and free peptides, and conjugates to a carrier molecule

The E1 and E2 domains drawn in gray were produced as a recombinant protein with an amino acid linker **(A)**, or as free synthetic peptides or coupled to the dextran carrier material **(B)**.

Fig. 12: Neutralizing antibodies after immunization with the recombinant ectodomain of p15E from FeLV

Goat 27 was immunized with the recombinant ectodomain, and the antiserum inhibited infection of FEA cells by feline leukemia virus, FeLV. The infection was detected by means of PCR (detection of provirus infection). **(A)** Titration of the virus with pre-immune serum, **(B)** titration of the virus with immune serum.

Fig. 13: Epitope mapping of FeLV-neutralizing serums

Following immunization with the recombinant ectodomain of p15 from FeLV, goat serum 27, 10 rat serums and antibodies purified using affinity chromatography were mapped as described in Fig. 3. Overlapping peptides corresponding to the ectodomain of p15 from FeLV were used. IgG: Prot G-purified antibodies, p15E - p15E-purified antibodies. The four most important epitope domains are framed.

Fig. 14: Effect of simultaneous immunization with recombinant protein p15E from FeLV and commercial Leucogen anti-FeLV vaccine

Rats were immunized with commercial Leucogen vaccine (animals 56.1, 56.2, 56.3) or with commercial Leucogen vaccine and recombinant protein p15E from FeLV. Neutralization was measured by means of quantitative real-time PCR, and this is illustrated as percent provirus integration. Clearly, the serums of group 54 FeLV (Leucogen and p15E) neutralize more favorably than serums of group 56 (Leucogen alone).

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